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THE EFFECTS OF MONOCHROMATIC ULTRAVIOLET RADIATION ON EGGS OF THE NEMATODE, *ENTEROBIUS VERMICULARIS*. II. SUBLETHAL EFFECTS

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We have described in a previous article (Hollaender, Jones and Jacobs, 1940) the quantitative response of the eggs of *Enterobius vermicularis* to seven wavelengths in the ultraviolet region between 2280Å and 2950Å, especially the wavelength dependence of the survival ratio for 50 per cent inactivation. We found a small maximum of sensitivity at 2805Å and increased response to ultraviolet radiation at wavelengths below 2400Å.

There will be described in this article (1) the effect of radiation on hatching rate; (2) a delayed lethal effect observed on eggs stored after irradiation; (3) the effect on the survival of larvae hatched from irradiated eggs; and (4) the effect of radiation on the further development of immature eggs.

In our method of irradiation, eggs in the ring-and-one-half stage, and in a few cases in the tadpole stage, were rotated in small Syracuse watch glasses in such a manner that the exposed dish received a measured quantity of monochromatic ultraviolet radiation and the control dish was protected against the ultraviolet. Most of the tests described in this article were conducted with wavelength 2650Å. A full description of these techniques is given in our previous article. The experiments reported here necessitated certain modifications in the techniques of testing effects of radiation, which will be described separately below.

EFFECT ON HATCHING RATE

A comparison was made of the relative percentage of total hatches of active larvae that were removed from the control and the irradiated dishes at successive observations during the four-hour hatching period. In control dishes, by far the greatest number were hatched in the first

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50-minute interval plus the time during which the larvae were being removed. Control and exposed dishes which had received relatively small quantities of radiation hatched at a similar rate. Also, occasionally a few dishes of material considered very resistant hatched similarly. However, most of the irradiated dishes which had received a considerable amount of energy showed a typical pattern of a slow start, followed by a more rapid hatching rate after the first hour with a drop during the last hour or during the last two observations.

Results of typical experiments are given in Table 1. A few of these

TABLE 1.—*Rate of hatching of control and of irradiated eggs*

Dish no.	Total no. larvae	Larvae removed at successive observations										Per cent hatch
		1		2		3		4		5		
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	
C 1 ...	71	65	96	3	4	..	..	..	..	..	..	96
C 1a ...	138	116	84	15	11	7	5	..	..	..	..	94
R 1 ...	157	130	83	15	9	7	4	5	3	..	..	97
R 2 ...	132	128	97	3	2	1	1	..	..	..	..	94
R 3 ...	22	1	5	5	23	6	27	9	41	1	5	30
R 4 ...	36	1	3	16	44	9	25	6	17	4	11	25
R 5 ...	4	2	50	1	25	1	25	..	..	..	..	3
C 2 ...	154	154	100	..	..	..	..	..	..	..	..	95
R 6 ...	61	33	54	16	26	2	3	10	16	..	..	40
R 7 ...	22	5	23	5	23	6	27	4	18	2	9	16
R 8 ...	18	0	...	3	17	6	33	5	28	4	22	13
C 3 ...	129	118	91	7	5	2	1	2	1	..	..	87
R 9 ...	28	3	11	3	11	13	46	7	25	2	7	19

Rate of hatch of control (C) and of irradiated eggs (R), as shown by numbers of active larvae recovered at successive observations during the 4-hour hatching period. Radiation was with wavelength 2380Å.

data are given in graphical form in Fig. 1. The per cent hatched is plotted against the respective number of observations. The broken line shows the hatching of controls, the solid lines that of two experiments in which there were 25 or 30 per cent survival. It is easily recognizable from these graphs that the control hatched almost 90 per cent by the time the first observation was completed. The irradiated material hatched only a very small percentage by the first observation, but an increasing hatch at the second observation, and at the fifth observation a few eggs were still hatching. This fifth observation took place at about four and one-half to five hours after irradiation. Apparently, with a sufficient amount of radiation, the rate of hatch of irradiated eggs is retarded. This retardation is shown also in Fig. 2, in which the percentage of the total hatch is plotted against the successive observations for R 9 and Control C 3.

EFFECT OF STORAGE ON ABILITY TO HATCH

The effects of radiation as determined by ability to hatch immediately after exposure are described in our previous paper. In addition it seemed necessary to test the possibility that effects of radiation might be

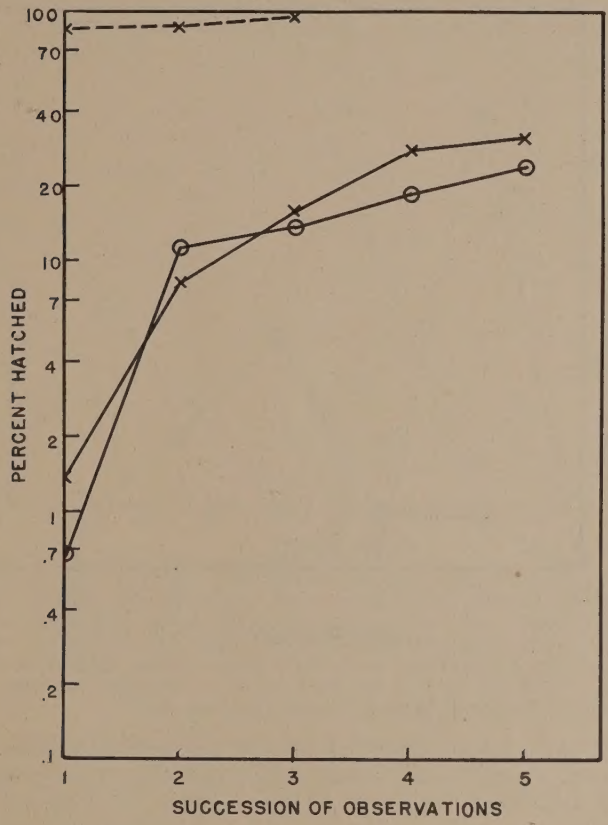

FIG. 1. Observation of percentage active larvae hatched during successive intervals of about 45 minutes. Broken line based on data from controls; solid line on data from irradiated eggs, in two experiments. See Table 1.

different if eggs were tested after storage subsequent to irradiation. Such differences in effects of radiation, considered as recovery and/or delayed effects, have been demonstrated in studies on other organisms (Hollaender and Duggar, 1938). To test these possibilities, experiments were set up as follows: A number of dishes of eggs were irradiated with the same amount of energy at the same wavelength. A control dish was prepared for comparison with each exposed dish. An irradiated dish and a control dish were tested at once for ability to hatch. The remaining exposed dishes were stored, with their controls, in covered Petri dishes, half of the dishes at 5° C and half at 24° to 26° C. An exposed and a control dish from those stored at each temperature were tested for

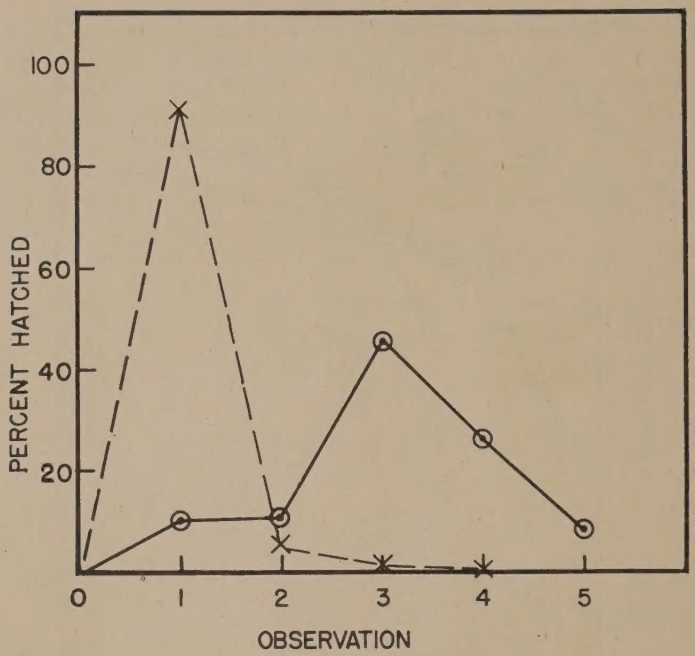

FIG. 2. Percentage of total active larvae, hatched during successive 45-minute intervals. Broken line based on data from controls (C 3); solid line on data from irradiated eggs (R 9) with 19 per cent hatch. See Table 1.

ability to hatch at approximately 18 and 40 hours, and in one case eggs were tested after 65 hours storage. Results of such experiments are given in Table 2 and Fig. 3.

Eggs in control dishes stored at 5° C kept their ability to hatch well, those stored at 24° to 26° C showed a drop in some cases but not in others. In general, normal eggs stored under these experimental conditions keep their ability to hatch to a fairly high percentage for several days. Eggs in irradiated dishes, as can be seen in column 6, Table 2, exhibit a very great drop in ability to hatch after 18 and 40 hours storage. Eggs which have survived considerable amounts of radiation will thus have a very marked increased dying rate when kept in storage only 18 hours. Apparently eggs which have received only small quantities of radiation will not show this striking mortality rate.

Fig. 3 shows the first set of data given in Table 2 in graphical form. The relative percentage of hatching is plotted against the hours of storage. This delayed effect of radiation, that is, the increased dying rate in storage is brought out most strikingly in this graph. On account of the importance of this delayed lethal effect, we repeated this experiment.

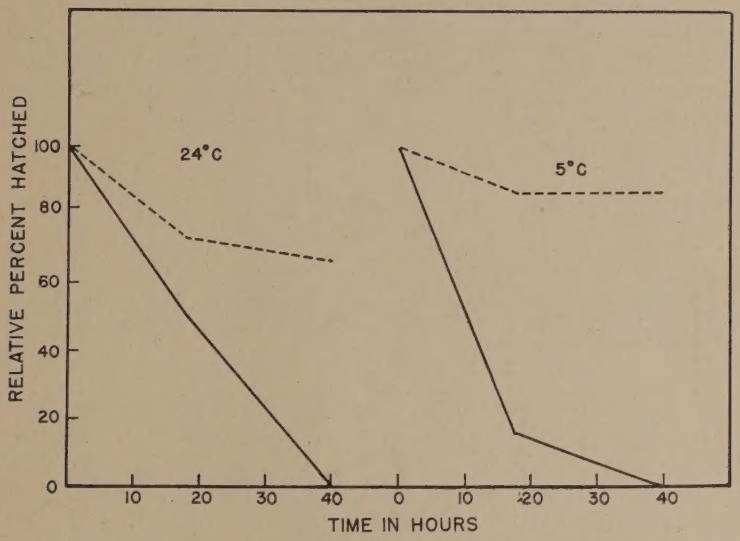

FIG. 3. Effect of storage after irradiation of eggs. Percentage of active larvae hatched from eggs tested immediately after storage up to 40 hours at (a) 24° C and (b) 5° C compared to controls and irradiated but unstored eggs. Broken lines based on data from controls; solid lines on data from irradiated eggs, with R–C survival ratio of 17 per cent.

EFFECT ON SURVIVAL OF LARVAE

Normal larvae after hatching will survive for a considerable time. In the course of testing the hatch in irradiated dishes, it appeared that larvae from irradiated eggs often did not survive in the digestive fluid for as long as comparable controls. It was somewhat difficult to check this point when testing a number of dishes at one time. However, incidental observations were made in a few of our experiments as follows: During the period of hatching, larvae were removed from experimental and control dishes and transferred into small Syracuse watch glasses containing physiological saline, kept at 37° C, and observed for activity at intervals of ½ to 1½ hours. A few data are given in Table 3. Fifty-four larvae, hatched as active larvae from an irradiated dish, had a survival after ½ to 1¼ hours of only 35 per cent. One hundred and nine larvae of the control which hatched as active larvae had a survival of 95 per cent after ½ to 1½ hours. Furthermore, it was observed that some of the active larvae hatching in irradiated dishes moved sluggishly and were presumably damaged even though able to hatch. Our data on this point are rather meager, but there is very little doubt that some larvae which have come from irradiated eggs given considerable energy are affected by the

TABLE 2.—*Modification of hatching by storage after irradiation*

Dish no.	Storage after irradiation		Total no. eggs	% active larvae	Relative percentage
	Time hours	Temp. °C			
			I		
C 1	0	..	162	95	100
C 2	18	24	152	70	73
C 3	40	24	151	63	66
R 1	0	..	137	16	100
R 2	18	24	144	8	52
R 3	40	24	150	0	0
C 4	18	5	137	82	86
C 5	40	5	153	84	87
R 4	18	5	151	3	16
R 5	40	5	150	0	0

The eggs in this experiment were irradiated with wavelength 2380 Å. The survival of the radiated as compared to the control eggs, the R–C survival ratio, is 17%. The *relative percentage* is the ratio of C and R dishes to C 1 and R 1 respectively, both of the latter being taken as 100%.

			II		
C 1	0	..	145	77	100
C 2	18	26	131	91	117
C 3	41	26	178	74	96
C 4	65	26	147	83	107
R 1	0	..	129	42	100
R 2	18	26	165	47	113
R 3	41	5	152	21	50
R 4	65	5	221	5	11
C 5	18	5	188	75	98
C 6	41	5	190	72	93
R 5	18	26	115	14	33
R 6	41	26	90	11	27

The eggs in this experiment were irradiated with wavelength 2650 Å. The R–C survival ratio in this case is 54%. Survival ratio and relative percentage are determined as above. The eggs in this experiment were floated on physiological saline instead of distilled water and were tested over a slightly longer hatching period.

radiation even though they are able to hatch as active larvae; that is, they exhibit an increased dying rate.

EFFECT ON IMMATURE EGGS

It is generally recognized that organisms in early stages of development are more susceptible to ultraviolet radiation than are more fully

TABLE 3.—*Survival of larvae hatched from eggs in one control and one irradiated dish*

Dish	Transfer dish no.	No. larvae placed in transfer dish	Time interval before observation (in hours)	No. active larvae in transfer dish
Control	1	85	1½	80
(77 per cent hatch)	2	15	1	15
	3	4	½	4
	4	5	¾	5
Totals		109		104
Irradiated	1	27	¾	9
(42 per cent hatch)	2	20	½	7
	3	3	¾	0
	4	3	1¼	3
	5	1	½	0
Totals		54		19

Radiation at wavelength 2650 Å.

developed ones. A few pinworm eggs with immature, tadpole-stage embryos were irradiated at 2650Å. Although only 3 dishes of eggs were used, these showed a definitely decreased ability to develop to ring-and-a-half infective stage larvae, when compared with eggs in control dishes, handled in a similar manner, but not exposed. Data on this experiment are given in Table 4. In order to prevent development before irradia-

TABLE 4.—*Development of tadpole-stage embryos after radiation at wavelength 2650 Å*

Energy ergs/egg	Time exposed minutes	Dish no.	Per cent developed	Per cent of controls developed
2.5	15	R 1	9	97
13.7	66	R 2	14	98
26.1	10	R 3	13	94

tion, the eggs used in this experiment were refrigerated at about 5° C, and it should be noted that the relatively high temperature of the laboratory may have allowed a little development of some of the eggs in the dishes exposed for the longer periods of time. However, the results indicate a definite inhibition of development of radiated eggs as compared with controls. Exact comparisons of susceptibility of tadpole and older embryos should be made on eggs of the same batch, but so far as our data allow comparison, these tadpole embryos appear to be about ten times more sensitive to radiation with 2650Å than ring-and-a-half or infective stage embryos. These results are in accord with the findings of other workers on the increase of resistance along with advancing development of nematode eggs (See Ruppert, 1924 and Shalimov, 1935).

DISCUSSION

A detailed consideration of the actual effects of monochromatic ultraviolet radiation on *Enterobius* eggs is difficult at the present time. Such a discussion must be based on a knowledge of the effect of the radiation on the various parts of the rather complicated nematode embryo and its coverings. As noted in our previous article, the membranes of the pinworm egg are three in number and composed of protein (outermost), chitin (middle), and a lipoid (innermost). In addition to these layers of the egg shell, it is assumed from general information on nematodes (Chitwood and Chitwood, 1937) that there are several layers of various proteins in the cuticula of the ring-and-a-half embryo itself. At this stage of development the embryo is fully differentiated except for the reproductive system, although growth occurs later. The various membranes of the pinworm egg, as well as the embryo itself, must be considered in our discussion of effects of radiation as determined by ability to hatch.

The process of hatching is apparently a combination of the digestion of the outermost protein layer and of the larva pushing its way through the lipoidal membrane of the shell at the hatching area, where the chitin is lacking. The actual emergence of the larvae with the rupturing of the lipoidal membrane can easily be observed. There may also be some stimulating effect of the digestive juice on the larva. This is the only work on radiation of nematode eggs known to us, in which hatching was used as the method of testing effects. Most work has been done on eggs which are not embryonated when laid and the criterion was their further development. For testing the effects of radiation on immature eggs we used, as did Shalimov, the further development of the tadpole embryos to the ring-and-a-half stage. However, our investigation was concerned mostly with ring-and-a-half stage eggs because from the standpoint of control these present the greatest problem. For determining the effects of radiation on these infective eggs the only criterion other than ability to hatch which could be used as a guide was the activity of the embryo within the egg, and this was at best a very inaccurate yardstick. Unfortunately, it is also impossible at present to follow the development of the pinworm through experimental animals, so that effects of radiation on later development could not be determined. Actually, ability to hatch, as a measure of effects of radiation is the logical test since the hatching and postembryonic development of the larva within the host follows attainment of the ring-and-a-half stage. However, it has its disadvantages since our method of artificial hatching involves a period of observation of at least four and a half hours. Such a long period before the effects of irradiation can be measured means that any early discontinuous effects might be missed.

There are several possibilities to consider in a discussion of radiation effects on the pinworm eggs. Since ultraviolet radiation will produce in certain proteins first a breaking down of macromolecules (Svedberg, 1939; Sanigar, Krejci and Kraemer, 1939) and finally coagulation of protein particles (Clark, 1935), it is probable that the outer protein membrane of the egg undergoes such changes due to radiation, as a result of which the permeability of the layer is changed and hatching may be affected. The innermost sterol membrane also might be affected with a change in its permeability. A change similar to that of the outermost membrane might also be brought about in the several thin layers of protein which form the cuticula of the embryo. Finally, some function of the embryo itself might be interfered with through a direct effect on the protoplasm. The actual coagulation of the embryo tissue has also been observed by Cole and Brackett (1939) who have photographed the egg in ultraviolet radiation.

One or more of the possible direct results of radiation on the pin-

worm egg membranes or embryo itself may be correlated with the sublethal effects described above. As already noted, any changes in the membranes and/or in their permeability might result in a retardation in the rate of hatch of irradiated eggs. We have observed that some eggs dried at known temperatures and humidities also exhibit a retarded hatch when tested subsequently; since very active embryos were observed in certain of these eggs, the delayed hatch in many might well be considered as due to a change in the membranes. This will be described more fully in a subsequent publication (Jones & Jacobs). On the other hand, since after irradiation with considerable energy some larvae which hatched were actually less active than controls, it is thought that damage to the embryo itself with its consequent lessened activity, also was a factor in retarding hatch.

The combined effect of storage and radiation is very detrimental to the pinworm egg. The storage effect on the controls is comparatively slight. The delayed lethal effect described above may be due simply to a gradual deterioration of the embryo after the production of the initial damage. It may be possible that in storage there is an increased sensitivity of the embryo to surrounding media which is emphasized by permeability changes in the protein and sterol membranes. Tissues of higher animals have been observed to be very sensitive after irradiation to detrimental surroundings (Hollaender and Wolf, unpublished data). Similar effects of change of sensitivity after irradiation have also been observed on micro-organisms like bacteria, fungous spores, etc (Hollaender & Duggar, 1938; Hollaender & Emmons, 1939). In all these cases irradiation changes the resistance pattern of these organisms. The literature on this point as concerns ultraviolet radiation is not very plentiful, but extensive material is available in connection with X-ray work (Colwell, 1935; Ray and Bodine, 1938).

The practical significance of these delayed radiation effects is important because irradiation in combination with other physical and chemical agents may help to destroy these eggs under practical conditions.

The low vitality observed in some larvae hatched from irradiated eggs is not surprising. The larva which is only slightly damaged by irradiation may have sufficient energy to leave the egg and move for a certain time after hatching. It may be that the larva has spent too much of its impaired energy in its escape from the egg, or that irradiation has inflicted cumulative damage so that it is unable to live more than a short time after hatching.

A possible explanation of the inability of the hatched larvae to live for an extended time after having received considerable amounts of radiation probably can be found in the discussion of Heilbrunn and co-workers with regard to the change in calcium content of tissues after irradia-

tion (Heilbrunn and Daugherty, 1933). Although we have no experimental proof on this point, it appears to us as a reasonable explanation.

All the membranes of the pinworm egg are present when the egg is laid in the tadpole stage. The greater sensitivity of tadpole stage embryos to ultraviolet radiation is therefore correlated with the stage of development of the embryo itself. Since the physiological factors involved in the attainment of the ring-and-a-half stage are not known, the precise mechanism affected by radiation of the tadpole stage which prevents or distorts development (see Shalimov) can not be stated.

Many of the points discussed here on the possible action of ultraviolet radiation can be checked experimentally. It is unfortunate that the pinworm egg is not more suitable for such tests, and it is thought that some different organisms which are more available and uniform will be more suitable.

SUMMARY

1. The rate of hatch is slower for eggs irradiated with monochromatic ultraviolet radiation given considerable energy than for controls.

2. A delayed lethal effect of ultraviolet radiation is indicated, since eggs stored after having been irradiated with a considerable amount of energy showed a smaller percentage hatch than others of the same batch which were tested immediately.

3. Many larvae which hatched from irradiated eggs survived for a shorter time than those hatching from eggs in control dishes.

4. Ultraviolet radiation is more damaging to immature eggs than to those which have attained the ring-and-a-half or infective stage.

5. The implications of these findings and the possible ways in which the above results have been produced are discussed.

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LIFE HISTORY STUDIES ON TWO STRIGEID TREMATODES OF THE DOUGLAS LAKE REGION, MICHIGAN[1]

LOUIS OLIVIER

The strigeid trematodes of the Douglas Lake Region, Michigan, have been the subject of research for more than two decades. Extensive studies on the cercariae, especially by Cort and his co-workers (1917, 1928, 1937, 1938, etc) have resulted in the description of eighteen species, including one whose life cycle is described here. Many metacercariae have been carefully described by Hughes (1927, 1928b, c, and d, 1929, etc).

The life cycles of six species of strigeids which occur in this region have been reported. Van Haitsma described the life cycles of *Cotylurus communis* (1930b), *Cotylurus flabelliformis* (1931a), and *Diplostomum flexicaudum* (1931b). Hunter (1933), Hunter and Hunter (1935), and Krull (1932, 1934) have demonstrated the life cycle of *Uvulifer ambloplitis*. *Cercaria burti* Miller, which occurs at Douglas Lake, has been shown by Willey and Rabinowitz (1938) to be a stage in the life cycle of *Apatemon globiceps*. Odlaug (in press) has described the life cycle of *Alaria intermedia*, the cercaria and mesocercaria of which are known to occur in the Douglas Lake Region. In addition, Ferguson (1936, 1937, 1938) and Hunter (1936, 1937) have described the life cycle of *Posthodiplostomum minimum* whose cercarial stage closely resembles *Cercaria physae* Cort and Brooks of the Douglas Lake Region.

The present account reports life history studies on those strigeid trematodes of the Douglas Lake Region whose metacercariae develop in tadpoles. The life histories of two such species have been completed experimentally. The first, *Diplostomum micradenum* (Cort and Brackett, 1938), was known previously only in the cercarial stage. The second, *Apharyngostrigea pipientis* (Faust, 1918), was known previously only in the metacercarial stage.

MATERIAL AND METHODS

The observations reported in this paper were made on material collected near the University of Michigan Biological Station on Douglas Lake, Michigan. Since the station operates only during the summer,

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[1] Contribution from the Biological Laboratory, New York University, University Heights. The writer wishes to express appreciation and gratitude to Dr. H. W. Stunkard and to Dr. W. W. Cort for numerous suggestions and criticisms throughout the course of the work. Thanks also are due to Dr. P. C. Beaver for assistance in several of the feeding experiments.

the periods of collection extended roughly from late June to the end of August.

To obtain larvae, snails and tadpoles were collected from Douglas Lake, Burt Lake, Black Lake, Munro Lake, and Lancaster Lake. For infection experiments, parasite-free tadpoles of *Rana pipiens* Schr., *Rana clamitans* Latr., and *Hyla versicolor* LeConte were taken from Vincent Lake, an acid lake in which snails are unable to live. No larval trematodes have ever been found in tadpoles taken from this lake.

The cercariae of *A. pipientis* were studied by methods described by Cort and Brackett (1937).

Living metacercariae of *D. micradenum* were obtained from both natural and experimental infections and were studied at all ages. More morphological data were obtained from living than from fixed and stained specimens. Young metacercariae of *A. pipientis* from experimental infections were studied alive to observe early stages of metamorphosis. Most of the information on the morphology of older metacercariae of *A. pipientis* was worked out from fixed material since living specimens more than eight or ten days old are opaque, probably because of the accumulation of cystogenous material.

Metacercariae and adults were dropped from a fine pipette into Gilson's fluid warmed to 60–65° C. This method is highly satisfactory because it fixes the worms in a relaxed condition and so retains normal body proportions. The use of pressure was avoided because it causes distortion, especially in adults. Mature metacercariae of *A. pipientis* were removed from their cysts before immersion in the fixative, since the thick cyst wall greatly impedes penetration of the fluid.

Metacercariae and adults intended for whole mounts were stained in paracarmine, Ehrlich's hematoxylin, Delafield's hematoxylin, or eosin. Sections of metacercariae and adults were stained in Heidenhain's hematoxylin and eosin or Mallory's triple connective tissue stain. The latter was very effective in delineating both larval and adult structures.

All measurements given in the text are from fixed specimens. It must be noted that measurements of metacercariae are unreliable for specific determination, because the worms vary greatly in size and shape according to their age, the degree of crowding, etc. Measurements of adults may also be very misleading unless variations caused by such factors as age of the worms, the condition of the host, species of host, fixative used, etc are considered.

In this report the generic and specific nomenclature used by Dubois (1938) has been followed.

REVIEW OF THE KNOWN LIFE HISTORIES IN THE STRIGEATA LA RUE, 1926

Von Linstow (1877), Leuckart (1889), Braun (1892) and others

believed that the miracidia of strigeids metamorphosed directly into metacercariae without the intervention of other larval stages. Accordingly, Leuckart regarded the strigeids as a group of "metastatic" trematodes which he placed between the Monogenea and the Digenea.

The true course of the strigeid life cycle was worked out by Lutz (1921), Ruszkowski (1922), Mathias (1922), and Szidat (1924a and b). (For a historical discussion see LaRue (1926) and Szidat (1929a).) The work of these men gave impetus and direction to life history studies on the strigeids. Subsequently, the completed life cycles for 24 species and partial life cycles for at least eighteen additional species have been reported. A list of those for which a substantial part or all of the life cycle has been reported appears, together with pertinent data, in Table 1. Names in parentheses in the columns listing first and second intermediate hosts refer to the cercarial and metacercarial stages of the parasites. Where many hosts have been reported for a particular life history stage, only a partial list is given. References cited include papers which describe larval stages as well as those which report results of life history studies. As noted in the tables, Szidat has suggested that the life history data presented for *Cotylurus communis* and *Cyathocotyle orientalis* may be incorrect. The validity of the life cycle of *Hysteromorpha triloba* as reported by Lutz is discussed elsewhere in this paper.

THE LIFE CYCLE OF *Diplostomum micradenum* (CORT AND BRACKETT)

Cort and Brackett (1938) described *Cercaria micradena* from a specimen of *Stagnicola palustris elodes* (Say) collected in 1937 at Black Lake, Cheboygan County, Michigan. The infected snail was turned over to me and experiments revealed that the cercariae penetrated tadpoles of *Rana pipiens* and developed into mature metacercariae identical with others previously taken from naturally infected tadpoles. Life history studies were continued during the summers of 1938 and 1939 and adults representing a new species of *Diplostomum* were obtained. The name for the species, therefore, becomes *Diplostomum micradenum* (Cort and Brackett, 1938). A brief report on the life cycle of this species was presented by Olivier (1938).

THE CERCARIA

Since the cercarial stage has been described fully (Cort and Brackett, 1938), no further account of its morphology and activity will be given.

Cercaria micradena appears to be rare in the Douglas Lake Region. Cort and Brackett reported one infection in a collection of 555 snails taken in the summer of 1937. In a group of 650 specimens of *Stagnicola palustris elodes* collected from the same locality in 1938, two were infected with *Cercaria micradena,* while among 1500 snails collected in 1939 from

TABLE 1

Specific Name	Final host	First intermediate host	Second intermediate host	References
		Family DIPLOSTOMIDAE		
Diplostomum spathaceum (Rud., 1819) Braun, 1898	*Larus argentatus* Pont. *Rissa tridactyla* (L.) *Alca tarda* L.	*Lymnaea stagnalis* (L.) *Lymnaea palustris* (O.F.M.) (*Cercaria C*, Szidat, 1924)	*Abramis blicca* (Bloch.) *Barbus barbus* (L.) *Phoxinus phoxinus* (L.) (*Diplostomulum volvens* (Nord.))	Szidat 1924a and b
Diplostomum flexicaudum (Cort and Brooks, 1928) Van Haitsma, 1931	*Larus argentatus* Pont.	*Stagnicola emarginata angulata* (Sow.) (*C. flexicaudum* C. and B.)	*Catostomus commersonii* (Lacép.) (*Diplostomulum gigas* H. and B.)	Cort and Brooks 1928 Hughes and Berkhout 1929 Van Haitsma 1931b
Diplostomum micradenum (Cort and Brackett, 1938) Olivier, this paper.	Domestic pigeon	*Stagnicola palustris elodes* (Say) (*C. micradena* C. and B.)	*Rana pipiens* Schr. *Bufo americanus* Hol.	Cort and Brackett 1938 Olivier 1938 Olivier, this paper.
Diplostomum huronense (LaRue, 1927) Hughes, 1929	*Larus argentatus* Pont.		*Percopsis omiscomaycus* (Walb.) *Perca flavescens* (Mitch.) (*Diplostomulum huronense* (LaRue) Hughes and Hall)	Hughes and Hall 1929 Van Haitsma 1931b
Posthodiplostomum minimum (MacCallum, 1921) Dubois, 1936	*Ardea herodias* L.	*Physa gyrina* Say (*C. multicellulata* Miller)	*Eupomotis gibbosus* (L.) *Ambloplitis rupestris* (Raf.) *Micropterus dolomieu* Lacép. (*Neascus van-cleavei* (Agers.))	Hughes 1928b Ferguson 1936, 1937, 1938 Hunter and Hunter 1940 Hunter 1936, 1937
Posthodiplostomum cuticola (Nordmann, 1832) Dubois, 1936	*Ardea cinerea* L. *Egretta garzetta* (L.) *Nycticorax nycticorax* (L.)		*Cobitis taenia* L. *Cyprinus carpio* L. *Perca fluviatilis* L.	Ciurea 1930
Tylodelphys excavata (Rud., 1803) Szidat, 1935	*Ciconia ciconia* L. *Nycticorax nycticorax* (L.)	*Planorbis corneus* (L.)	*Rana esculenta* L. *Rana temporaria* L. (*Diplostomulum rhachiacum* (Hent.))	Ciurea 1928 Szidat 1935
Tylodelphys clavata (Nordmann, 1832) Diesing, 1850	*Circus aeruginosus* (L.) *Ardea cinerea* L.		*Perca fluviatilis* L. *Abramis brama* (L.) *Leuciscus idus* (L.) (*Diplostomulum clavatum* (Nord.))	Ciurea 1928
Ornithodiplostomum ptychocheilus (Faust, 1917) Dubois, 1936	*Mergus americanus* Cassin *Mergus serrator* L. Domestic Duck		*Ptychocheilus oregonensis* (Rich.) (*Neascus ptychocheilus* (Faust))	Hughes and Piszczek 1928 Van Haitsma 1930a
Neodiplostomum lucidum LaRue and Bosma, 1927	*Didelphis virginiana* Kerr.	*Physa ampullacea* Gould		West 1935
Neodiplostomum perlatum (Ciurea, 1911) Ciurea, 1929	*Haliaeetus albicilla* (L.) *Circus aeruginosus* (L.) *Falco subbuteo* L.		*Cyprinus carpio* L.	Ciurea 1929
Crassiphiala bulboglossa Van Haitsma, 1925	*Ceryle alcyon* (L.)		*Perca flavescens* (Mitch.) (*Neascus bulboglossa* (Hughes))	Van Haitsma 1925 Hughes 1928c

TABLE 1.—*Continued*

Specific name	Final host	First intermediate host	Second intermediate host	References
		Family DIPLOSTOMIDAE (*Continued*)		
Uvulifer ambloplitis (Hughes, 1927) Dubois, 1938	*Ceryle alcyon* (L.)	*Helisoma trivolvis* (Say) *H. campanulatum* (Say) (*C. bessiae* Cort and Brooks)	*Ambloplitis rupestris* (Raf.) *Micropterus dolomieu* Lacép. *Eupomotis gibbosus* (L.) (*Neascus ambloplitis* Hughes)	Hughes 1927 Cort and Brooks 1928 Hunter and Hunter 1930, 1935 Hunter 1933 Krull 1932, 1934
Alaria mustelae Bosma, 1931	*Mustela vison* Schr. *Ondatra zibethica* (L.) Cat, dog	*Planorbula armigera* (Say)	Mesocercariae in *Rana pipiens, Rana clamitans* Metacercariae in *Peromyscus leucopus noveboracensis* (Fischer) Rat, mouse	Bosma 1931, 1934
Alaria intermedia (Olivier and Odlaug, 1938) Odlaug, Thesis	Cat Dog	*Planorbula armigera* (Say)	Mesocercariae in *Rana pipiens* Schr. Metacercariae in Rat, mouse	Olivier and Odlaug, 1938 Odlaug (in press) Thesis
Alaria alata (Goeze, 1782) Krause, 1914	*Canis lupus* L. Cat, dog *Vulpes vulpes* (L.)	*Planorbis vortex* L.		Ruszkowski 1922
? *Alaria* sp.		*Helisoma trivolvis* (Say) *H. campanulatum* (Say) (*C. marcianae* Cort and Brooks)	Mesocercariae in *Rana pipiens* Schr.	Cort 1918 Cort and Brooks 1928
Bolbophorus confusus (Krause, 1914) Dubois, 1935	*Pelecanus crispus* Bruch *Pelecanus erythrorhynchus* Gm. *Pelecanus onocrotalus* L.		*Abramis brama* (L.) *Perca fluviatilis* L. *Esox lucius* L.	Ciurea 1933
Pharyngostomum cordatum (Diesing, 1850) Ciurea, 1922	Cat	*Segmentina calathus* Benson	*Bufo melanostictus* *Rana limnocharis* (*Diplostomulum mutadomum* Wallace)	Wallace 1937, 1939
Hysteromorpha triloba (Rudolphi, 1819) Lutz, 1931	*Herodias egretta* (Gm.) *Nyctanassa violacea* (L.)	*Planorbis immunis* Lutz	*Poecilia vivipara* Bl. Sch. *Leptodactylus ocellatus* (L.) *Abramis balterus* (L.) (*Diplostomulum trilobum* (Rud.))	Lutz 1933 Ciurea 1930, 1933
		Family PROTERODIPLOSTOMIDAE		
Proalarioides serpentis Yamaguti, 1933	*Elaphe quadrivirgata* (Boie) *Elaphe climacophora* (Boie)		*Rana nigromaculata* Hallowell	Yamaguti 1933, 1936

TABLE 1.—*Continued*

Specific name	Final host	First intermediate host	Second intermediate host	References
		Family STRIGEIDAE		
Strigea strigis (Schrank, 1788) Abild., 1790	*Asio flammeus* (Pont.) *Strix pygmeae* Bechst. *Buteo buteo* (L.)		*Tropidonotus natrix* L. (*Tetracotyle colubri* Linst.)	Braun 1894
Strigea vaginata (Brandes, 1888) Szidat, 1928	*Cathartes urubitinga* Pelz. *Spizaetus ornatus* (Daud.)	*Spirulina anatina* *Spirulina mellea* (*C. gyrinipeta* (Lutz))	Mesocercariae in *Leptodactylus ocellatus* (L.) *Hyla crepitans* Wied. Metacercariae in pigeons, fish, white mice	Lutz 1933, 1934
Apharyngostrigea ibis Azim, 1935	*Ardeola ibis ibis* (L.)	*Planorbis boissyi* Pot. & Mich. *Bullinus dybowskyi* Fisch. *Pyrgophysa forskali* (Ehrenb.)	*Bufo vulgaris* Laur.	Azim 1935
Apharyngostrigea pipientis (Faust, 1918) Olivier, this paper	Domestic pigeon	*Planorbula armigera* (Say)	*Rana pipiens* Schr. *Hyla versicolor* LeConte (*Tetracotyle pipientis* Faust, 1918)	Faust 1918 Hughes 1928a Olivier 1939a and b Olivier, this paper
Cotylurus cornutus (Rud., 1808) Szidat, 1928	*Charadrius apricarius* L. *Vanellus vanellus* (L.) Domestic duck	*Lymnaea palustris* (O.F.M.) *Lymnaea stagnalis* (L.) *Lymnaea limosa* (L.) (*Cercaria A* Szidat)	*Lymnaea palustris* (O.F.M.) *Herpobdella atomaria* Car. (*Tetracotyle typica* Dies.)	Lutz 1921 Mathias 1922, 1925 Szidat 1924a, 1929c
Cotylurus flabelliformis (Faust, 1917) Van Haitsma, 1931	*Anas rubripes* Brewst. *Marila affinis* (Eyton) *Dafila acuta* (L.) *Spatula clypeata* (L.)	*Stagnicola emarginata angulata* (Sow.) *Lymnaea stagnalis* (L.) (*C. douglasi* Cort)	*Stagnicola emarginata angulata* (Sow.) *Lymnaea stagnalis* (L.) (*Tetracotyle flabelliformis* (Faust))	Cort 1917 Van Haitsma 1931a Hughes 1929
Cotylurus communis (Hughes, 1928) LaRue, 1932	*Larus argentatus* Pont.	*Stagnicola emarginata angulata* (Sow.) (*C. emarginatae* Cort)	*Percopsis omiscomaycus* (Walb.) *Catostomus commersonii* (Lac.) (*Tetracotyle communis* Hughes)	Cort 1917 Hughes 1928d Van Haitsma 1930b Szidat (1931) questions correctness of this cycle.
Cotylurus pileatus (Rud., 1802) Dubois, 1937	*Larus argentatus* Pont. *Sterna hirundo* L. *Sterna paradisaea* Brünn.		*Perca fluviatilis* L. *Esox lucius* L. (*Tetracotyle ovata* Linst.)	Braun 1894 Hughes 1928d
Apatemon gracilis (Rud., 1819) Szidat, 1928	*Anas platyrhynchos* L. *Mergus merganser* L.	*Bithynia tentaculata* (L.)	*Herpobdella atomaria* Car. *Haemopis sanguisuga* L.	Szidat 1929c, 1931
Apatemon fuligulae Yamaguti, 1933	*Fuligula fuligula* (L.) Domestic duck		*Parasilurus asotus* (L.) *Pseudobagrus aurantiacus* (Temm. and Schleg.)	Yamaguti 1933
Apatemon pellucidus Yamaguti, 1933	Domestic duck		*Mogurnda obscura* (Temm. and Schleg.)	Yamaguti 1933
Apatemon globiceps Dubois, 1937	Domestic duck	*Helisoma antrosum* (Con.) (*C. burti* Miller)	*Herpobdella punctata* (Leidy)	Willey and Rablnowitz 1938 Miller 1926

TABLE 1.—*Continued*

Specific name	Final host	First intermediate host	Second intermediate host	References
		Family Cyathocotylidae		
Prohemistomum vivax (Sonsino, 1892) Azim, 1933	*Milvus migrans aegyptius* (Gm.) Dog	*Cleopatra bulimoides* Jick. (*C. vivax* Sonsino, 1892)	*Gambusia affinis* (Baird and Girard) *Tilapia nilotica* (L.)	Azim 1933
Szidatia joyeuxi (Hughes, 1929) Dubois, 1938	*Tropidonotus natrix* L. *Tropidonotus viperinus* (Lat.)	*Cleopatra bulimoides* Jick. (*C. vivax* Sonsino, 1894)	*Rana esculenta* L. *Rana ridibunda* Pall. *Astatotilapia desfontainesi* (Lac.) (*Diplostomulum joyeuxi* Hughes)	Joyeux 1923 Joyeux and Baer 1934
Linstowiella viviparae (Linstow, 1877) Szidat, 1933	*Sterna paradisaea* Brünn. White rat	*Vivipara vivipara* L. (*C. monostomi viviparae* Szidat)	*Vivipara vivipara* L.	Szidat 1933, 1936
Mesostephanus odhneri (Travassos, 1924) Lutz, 1935	*Nyctanassa violacea* (L.)		*Haemulon* sp.	Travassos 1924
Mesostephanus appendiculatus (Ciurea, 1916) Lutz, 1935	Dog Cat		*Aspius aspius* (L.) *Abramis blicca* (Bloch.) *Tinca tinca* (L.)	Ciurea 1916
Cyathocotyle gravieri Mathias, 1935	Domestic duck	*Bithynia tentaculata* (L.)	Gudgeons and minnows	Mathias 1935
Cyathocotyle orientalis Faust, 1922	Domestic duck		*Vivipara lapillorum* Heude (*Tetracotyle orientalis* Faust)	Faust 1922 Questioned by Szidat 1929a
Cyathocotyloides curonensis (Szidat, 1933) Szidat, 1936	Domestic duck	*Bithynia tentaculata* (L.)	*Leucaspius delineatus* (Heck.)	Szidat 1933, 1936
Paracoenogonimus ovatus Katsurada, 1914	Mouse	*Vivipara vivipara* L.	"Goldorfen" (*Leuciscus idus* (L.))	Komiya 1938

that region only one infected snail appeared. Six hundred specimens of *S. palustris clodes* collected in 1939 from another locality were uninfected.

PENETRATION EXPERIMENTS WITH *Cercaria micradena*

Since early experiments revealed that *Cercaria micradena* penetrates tadpoles of *Rana pipiens,* large numbers of these tadpoles were infected over a period of three summers. The cercariae are not attracted to the tadpoles but when chance contact is made they attach by their suckers, creep about, and finally penetrate the skin with the aid of their spines and penetration glands. Before penetration the cercariae bend sharply several times at the junction of the body and tail and then cast off the tail. After penetration the cercariae migrate to the central nervous system of their host. The migratory path was not determined but almost all of them reach their destination within a few days. Sections of tadpoles infected for six weeks reveal that the larvae are unencysted and occur within the cavities of the brain and spinal cord or under the meninges (Figs. 34 & 35).

Attempts to infect metamorphosed specimens of *R. pipiens* were made on several occasions. All were unsuccessful and it is concluded that infection does not occur after metamorphosis. Tadpoles of *Triturus viridescens viridescens* Raf. were not available and attempts to infect adult specimens failed.

One tadpole of *Hyla versicolor* was exposed to cercariae and a number of very small metacercariae resembling the cercarial body were recovered the next day. No metacercariae of this species were found in a limited number of adults of *H. versicolor* collected in the field. In another experiment ten tadpoles of *R. clamitans* were exposed to *Cercaria micradena.* Two, which were dissected on the following day, contained minute larvae indicating that the cercariae had penetrated successfully. Three weeks later the eight remaining tadpoles were dissected. In seven of them no metacercariae were found while in the eighth there were four very small larvae. They had undergone very little development after penetration and probably would not have developed to maturity. More extensive studies are needed but it seems likely that, although cercariae of this species may penetrate tadpoles of both *Hyla versicolor* and *Rana clamitans,* they cannot complete their development in these hosts.

DEVELOPMENT IN TADPOLES

Living larvae were utilized to study early morphological changes in the metacercariae of *D. micradenum.* On July 15, 1938, eight tadpoles were infected with cercariae from one snail. On each day thereafter one tadpole was killed and the metacercariae studied. Figs. 1 to 5 illustrate some of the day by day changes observed, and summarized observations

on the larvae during the eight-day period are given below. It must be pointed out that the development in this series was relatively slow, probably because environmental temperatures were low. Development was more rapid in tadpoles infected in warmer periods. (Fig. 6).

Development During the First Four Days

During the first four days after penetration the oral sucker, ventral sucker, digestive system, and primary excretory system remained essentially unchanged. The constriction behind the ventral sucker and the glands were lost. On the third day the beginnings of the reserve excretory system appeared as variously shaped buds from the anterior portions of the bladder arms and developed only slightly during the fourth day. Ten fixed specimens from the four-day infection averaged 0.18 by 0.06 mm.

Development During the Second Four Days

All the main reserve excretory channels were begun during this four-day period. Their method of formation may be traced on Figs. 1 to 6. It should be noted that the main channels do not always form in exactly the same way and that the number and position of the more distal channels vary. There was no increase in the flame cell number at the sixth day (Fig. 4) and probably none at the eighth day. The oral sucker changed considerably during the sixth and seventh days, and after eight days began to resemble that of the mature metacercariae (Figs. 5 & 6).

Later Development

Metacercariae from another series of experimentally infected tadpoles furnished data on later developmental changes. A group of tadpoles was infected on July 9, 1938, and, beginning ten days later, one tadpole was sacrificed and its metacercariae examined each day for thirteen days.

Ten-day larvae of this series were very much farther advanced than the eight-day larvae of the first series and had attained a size of 0.35 by 0.15 mm (average of ten fixed specimens). There was a small hind-body on the postero-dorsal portion of the fore-body. The oral sucker had completed its metamorphosis and rudiments of the paired lateral sucking cups were observed on either side of the oral sucker. The holdfast had already begun to form and possessed a median ventral depression. All of the main channels and many of the smaller branches of the reserve excretory system were completed although there were no excretory concretions. Tiny concretions made their appearance in the ends of the reserve excretory channels on the 13th day. On the 14th day the largest concretions were 3 μ in diameter and on the 16th day were about 4.5 μ in diameter. In 23-day larvae they ranged in size from 2.5 to 7.5 μ.

The 23-day metacercariae had the size and all other morphological

characteristics of mature metacercariae and appeared to have completed metamorphosis. Experiments, however, indicated that they are not infective until much later.

MATURE METACERCARIA

(Figs. 7, 8, 33)

Small numbers of mature metacercariae have been found in tadpoles of *Bufo americanus* Hol., and in tadpoles and adults of *Rana pipiens* taken in the Douglas Lake Region. They have also been raised experimentally in *Rana pipiens* tadpoles. The mortality of heavily infected tadpoles is high but more study is required to determine how and to what extent the parasites injure their hosts.

The metacercariae are typical strigeid diplostomula with lateral sucking cups and an elongate hind-body. When not subjected to pressure, the fore-body is oval and somewhat concave beneath. The elongate hind-body bears the excretory pore at its tip. The positions of the muscular lateral sucking cups are usually marked by prominences on the margin of the fore-body. Twenty-five fixed specimens measured 0.34–0.45 mm long and 0.20–0.25 mm broad. The oral sucker of ten fixed specimens measured 34–47 μ in diameter and the ventral sucker, which lies just posterior to the middle of the fore-body, 25–43 μ in diameter. There is a short prepharynx and a relatively large pharynx which is elongate in most living specimens but may be ovoid or spherical in fixed ones. The pharynx of ten fixed specimens measured from 18–22 μ in diameter. The inconspicuous ceca, which originate about half way between the pharynx and the ventral sucker, are ventral and extend into the hind-body. The anlage of the holdfast organ, lying behind the ventral sucker, has a small cavity opening ventrally. Both the ventral sucker and the holdfast may be protruded.

The reserve excretory system, which is extensive and tends to obscure other structures, is shown in Fig. 7. The excretory bladder is V-shaped and occupies most of the hind-body. At the level of the holdfast, the bladder arms receive the common collecting tubules of the primary excretory system. The arrangement of the large longitudinal and transverse channels is constant and typical but many smaller branches vary in number and position. In the slightly enlarged end of each minute branch of the system there is a calcareous concretion. Most of these bodies are approximately spherical but some may have other shapes.

Of the many diplostomula which have been described, five species occur in or about the central nervous system of their hosts: *Diplostomulum petromyzi-fluviatilis* (Diesing) from ammocoetes larvae, *D. phoxini* (Faust) from *Phoxinus laevis* and *P. phoxinus, D. craniarum* (Diesing) from *Cobitis fossilis, D. rhachiaeum* (Henle) from *Rana esculenta* and *R. temporaria,* and *D. trituri* Kelley from *Triturus viridescens viridescens.* It is difficult to determine the specific identity of strigeid metacercariae because they have few characteristics upon which to base distinctions and because many have been inadequately described. Therefore, although the metacercaria of *D. micradenum* is considered distinct from these species, it may be conspecific with one or more of them. Of the five species listed above only *D. rhachiaeum* and *D. trituri* have been reported from amphibia. Szidat (1935) showed that the former develops into *Tylodelphys excavata* in the stork, *Ciconia ciconia* L. Although Kelley's (1934)

description is too incomplete to permit adequate comparison, the metacercaria of *D. micradenum* appears to differ from *D. trituri* in the shape of the hind-body and sizes of the suckers and pharynx. Experiments to test the identity of *D. micradenum* and *D. trituri* were prevented because, as noted earlier, tadpoles of *Triturus viridescens viridescens* were not available.

ADULTS

(Figs. 9 to 13)

On September 9, 1938, 123 mature diplostomes were recovered from the small intestine of a domestic pigeon which had been fed two infected tadpoles ten days earlier. The tadpoles harbored metacercariae that were 51 days old. Other experimental pigeons were fed larvae 14, 18, 20, 21, 27, 30, 35, 37, 39, 41, 43, and 55 days old but no additional adults were recovered. It is believed that most of these pigeons failed to become infected because the metacercariae were immature. Only one of the unsuccessful feeding experiments with pigeons involved metacercariae more than 43 days old. In that case a tadpole exposed to cercariae 55 days before was used. There appears to be no satisfactory explanation for the failure to secure adults in that experiment.

It is known that some strigeid metacercariae require a long period of development before they become infective. Van Haitsma (1931b) suggested that the diplostomula of *Diplostomum flexicaudum* probably mature in five or six weeks. According to Bosma (1934), the metacercariae of *Alaria mustelae* require nine weeks to mature. Azim (1935) stated that the metacercariae of *Apharyngostrigea ibis* do not encyst until the fifth week. Willey and Rabinowitz (1938) reported that metacercariae which develop from *Cercaria burti* Miller encyst in the leech 35–40 days after penetration. Probably they are not infective until some time after encystment.

Undoubtedly the pigeon is not a field host for *Diplostomum micradenum*. Attempts to determine the host or hosts for this species in the field have been unsuccessful.

Specific Diagnosis: Characteristics of the genus: Small worms. Total length 1.04–1.35 mm, mean 1.15 ± 0.09 mm.[2] Fore-body round to oval, concave ventrally, slightly shorter than the hind-body. Length of fore-body 0.46–0.63 mm, mean 0.54 ± 0.04 mm; width 0.51–0.61 mm, mean 0.55 ± 0.04 mm. Hind-body narrower than fore-body and usually set off from it by a slight constriction. Length of hind-body 0.52–0.71 mm, mean 0.61 ± 0.07 mm; width 0.38–0.52 mm, mean 0.45 ± 0.04 mm. A pair of muscular "lateral organs" situated ventro-laterally, well separated from oral sucker. Ventral sucker about in middle of fore-body, usually depressed below the surface and often obscured by anterior margin of the holdfast organ. Antero-posterior diameter of ventral sucker (17 specimens) 0.06–0.11 mm, mean 0.08 mm. Holdfast organ with circular contour, about half the width of the fore-body when everted.

[2] Measurements, unless otherwise stated, are from 32 specimens. The mean and its probable error are given to indicate the character of the variation.

Cavity of the holdfast irregular and the opening usually elongated antero-posteriorly. A spineless area about the lateral sucking cups and between them ventrally. Cuticular spines cover the rest of the fore-body ventrally and extend back to the acetabular level dorsally. Spines on the posterior ventral portion of the fore-body are bifid or occasionally trifid. Surface of the holdfast and external portions of its cavity covered with spines. Spines in the common genital duct and deeper parts of the genital atrium. Oral sucker diameter (15 specimens) 0.07–0.09 mm, mean 0.08 mm. Pharynx posterior and dorsal to oral sucker; diameter (16 specimens) 0.06–0.08 mm, mean 0.06 mm. Esophagus short, ceca dorso-lateral in fore-body, ventro-lateral in hind-body. Vitellaria always extend anterior to the ventral sucker when the latter is not protruded. Vitellaria predominately ventral in hind-body. Anterior testis (testis I) lateral, entire, asymmetrical; length (18 specimens) 0.15–0.22 mm, mean 0.17 mm; width (17 specimens) 0.14–0.25 mm, mean 0.19 mm. Posterior testis (testis II) large, horseshoe shaped; length (17 specimens) 0.13–0.19 mm, mean 0.15 mm; width (17 specimens) 0.21–0.36 mm, mean 0.29 mm. Ovary ovoid, median, just anterior to testis I. Ovary length (17 specimens) 0.07–0.10 mm, mean 0.09 mm; width (18 specimens) 0.10–0.14 mm, mean 0.12 mm.

Habitat: Small intestine.

Host: Domestic pigeon (experimental).

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 36701.

Male Reproductive System

A narrow vas efferens arises from the median anterior surface of testis I and becomes dilated before uniting with the vas efferens from testis II to form the vas deferens just anterior to Mehlis' gland. The vas efferens from the posterior testis is narrow and arises from the anterior surface at the base of the ventral groove. As it runs forward it bends laterally and becomes greatly dilated at the level of testis I. The vas deferens proceeds posteriorly in a path immediately dorsal to the uterus. Behind the posterior testis it again expands to form the thin-walled seminal vesicle from which the short and narrow ejaculatory duct leads to the genital atrium.

Female Reproductive System

The ovary is usually median just anterior to the level of testis I. The oviduct arises from the postero-dorsal surface of the ovary and almost immediately gives off Laurer's canal which opens dorsally near the mid-line at the ovarian level. The oviduct then turns posteriorly and, after receiving the common vitelline duct, enters Mehlis' gland. The uterus passes forward to the anterior end of the hind-body, then turns and runs posteriorly along the mid-line ventral to the other organs, to the genital sinus. The right and left vitelline ducts unite to form the large vitelline reservoir immediately posterior to Mehlis' gland. The common vitelline duct is narrow and opens into the oviduct at some distance from the ovary.

Amphitypy

The reproductive organs of *D. micradenum* are amphitypic. In ten specimens in which this characteristic could be observed, the anterior testis

was on the left in four and on the right in six. Other structures are reversed correspondingly. Amphitypy was described for *D. spathaceum* by Krause (1914) and for *D. flexicaudum* by Van Haitsma (1931b).

TAXONOMIC RELATIONS

Dubois (1938) included nineteen species in the genus *Diplostomum* (two of them in "Notes Additionnelles").[3] Four of these species, *D. alarioides* Dubois, *D. colymbi* (Dubois), *D. pelmatoides* Dubois, and *D. pusillum* (Dubois) have sucker-like lateral cups well separated from the oral sucker and in this respect resemble *D. micradenum*. The latter may be readily distinguished from *D. alarioides* and *D. pelmatoides* because both of these species have an acetabuliform bursa copulatrix. *D. micradenum* is similar morphologically to *D. pusillum* and *D. colymbi*. In both of these species the vitellaria extend forward only to the middle of the ventral sucker, whereas in *D. micradenum* the vitellaria always extend to the anterior margin of the ventral sucker and usually beyond this level when the acetabulum is not protruded. In addition, *D. pusillum* seems to be smaller than *D. micradenum* while *D. colymbi* appears to be larger. Subsequent life history studies may demonstrate that *D. micradenum* is conspecific with one or both of these species. Meanwhile, in the absence of conclusive evidence, it is considered preferable to add a species which later may be dropped into synonymy than to confuse two separate species in a common description.

THE LIFE CYCLE OF *Apharyngostrigea pipientis* (FAUST)

Five specimens of *Planorbula armigera* (Say) collected from a beach pool on the shore of Douglas Lake in the summers of 1938 and 1939 were infected with strigeid cercariae strikingly different from species previously encountered in the region. These cercariae penetrated tadpoles of *Rana pipiens* and developed into encysted metacercariae which were identified as *Tetracotyle pipientis* Faust, 1918. When fed to domestic pigeons, these metacercariae became adults which could be referred to the genus *Apharyngostrigea* but to none of the described species. The specific name of the metacercariae, therefore, is valid and the name for the species becomes *Apharyngostrigea pipientis* (Faust, 1918). A brief report on the life cycle of this species was presented by Olivier (1939b).

THE CERCARIA

Among 625 specimens of *P. armigera* collected on July 25 and July 27, 1938, four were infected with the cercaria of *A. pipientis*. On June 15, 1939, one infected snail appeared among 85 collected. No additional infections were encountered in 1428 snails collected throughout the remainder of that season.

[3] In a brief note, McIntosh (1939) added *D. fosteri* which appears to differ from *D. alarioides* in the distribution of the vitellaria.

Specific Diagnosis of the Cercaria (Figs. 14 to 18, 31): Strigeid cercaria. Length of body 0.093–0.174 mm, mean 0.134 ± 0.002 mm; width 0.050–0.081 mm, mean 0.057 ± 0.0006 mm.[4] Broad contractile tail stem, bulging at the middle, with large fluid-filled cavity. Tail stem length 0.174–0.391 mm, mean 0.298 ± 0.005 mm; width at broadest point 0.080–0.180 mm, mean 0.130 ± 0.002 mm. Furcae broad, flat, contractile. Furcal length (46 worms) 0.118–0.229 mm, mean 0.184 ± 0.002 mm. Oral sucker length 0.036–0.059 mm, mean 0.044 ± 0.0004 mm; width 0.027–0.045 mm, mean 0.031 ± 0.0004 mm. Ventral sucker behind middle of body; diameter 0.018–0.027 mm, mean 0.023 ± 0.0002 mm. Oral opening terminal, prepharynx short, pharynx distinct, small. Esophagus reaches about half way from pharynx to ventral sucker. Intestinal ceca inconspicuous, extend almost to level of genital primordium. Four pairs of small penetration glands grouped variously about ventral sucker, usually with four glands before and four lateral to or behind the sucker. The gland ducts on each side run anteriorly, penetrate the oral sucker in which they are dilated, and open terminally lateral to the oral opening.

Excretory system characteristic of strigeids. Bladder small. Two pairs of flame cells on each anterior collecting tubule. On each posterior collecting tubule two pairs in the body and one pair on each side in the proximal portion of the tail. Two transverse commissures, one behind the pharynx, the other posterior to the ventral sucker. A band of eight to ten irregular rows of closely set spines surrounds a circum-oral spineless area. Behind these rows, scattered spines over most of the body. Spineless areas behind ventral sucker and on mid-dorsal surface. A few spines on base of tail. Single irregular row of small spines on ventral sucker. No forward projecting spines. Hair-like projections on body and tail stem. A pair of small, lateral "unpigmented eye-spots" anterior to ventral sucker. "Island of Cort" not observed. Cercariae develop in small elongate sporocysts with slightly protruded birth pore and short birth canal (Fig. 19).

Host: Planorbula armigera (Say).

Locality: Beach pool, Douglas Lake, Cheboygan County, Michigan.

When undisturbed the cercariae exhibit activity similar to that of most other strigeids. They are distributed uniformly throughout the container and the majority hang quietly with the body contracted, the tail stem straight and elongate, and the furcae separated by an angle of not more than 60 degrees. The cercariae sink gradually and from time to time swim upwards, tail foremost, for several times their length, but only a small percentage are swimming at any one time. While the cercariae swim the body and tail stem are extended and the furcae are held at right angles to the latter. The cercariae of *A. pipientis* swim more slowly than those of other strigeids but they are propelled by the same "vibratory motion." There is a node at either end of the tail stem about which the vibration occurs. This motion is induced by alternate contractions of the longitudinal muscle fibers on either side of the tail stem which cause the latter to bend first to one side and then to the other. The furcae, which are whipped back and forth by the bending of the tail stem, pull the cercaria through the water tail foremost.

The tail of this species is unusual both structurally and functionally. The furcae are broad and are flattened laterally. The tail stem is thick at the middle and tapers toward either end. Beneath the thin, elastic

[4] Unless otherwise noted, measurements are from fifty worms. The mean and its probable error are included to indicate the character of the variation.

cuticula there are circular muscle fibers which are so spaced that under certain conditions of contraction they cause regular annulations in the wall of the tail stem. Dorsally and ventrally they interlace in a characteristic fashion (Fig. 15). Beneath these annular fibers there are two longitudinal rows of cells which extend along the mid-dorsal and mid-ventral lines (Fig. 15), and four groups of longitudinal muscle fibers, which originate at the base of the tail and are inserted in the walls of the furcae (Figs. 15 and 17).

An excretory tubule passes backward from the bladder, traverses the spacious cavity of the tail stem, and bifurcates to form two branches which open on the dorsal edges of the furcae about half way to their tips. Large cells with homogeneous cytoplasm and clear nuclei are applied closely to the excretory tubule from its basal end to the bifurcation. These cells, which probably correspond to the "caudal bodies" described for many strigeids, are connected by protoplasmic processes to cells on the wall of the tail stem. The large residual space in the stem and furcae contains a clear, watery fluid.

As stated previously, when the cercariae are unmolested their activity does not differ appreciably from that of most other strigeid cercariae. Under abnormal conditions, however, their activity is striking and unusual. The tail stems of typical strigeids, although they possess both longitudinal and circular muscles, are able to contract only very slightly under pressure and their sides always remain parallel. On the other hand, when a cercaria of *A. pipientis* is subjected to contact stimuli the tail stem and furcae undergo longitudinal contraction accompanied by lateral bulging of the walls of the tail stem so that its contour may become approximately circular (Fig. 14). The lateral expansion accommodates the fluid displaced by the contraction of the tail stem and furcae. The slight restraining action of the circular muscles of the tail stem causes the contour to become scalloped. When contracted completely, the tail stem has depressions anteriorly and posteriorly which partially envelop the body and the furcae respectively.

Lutz (1931) described a species of strigeid cercariae very similar to, and possibly identical with, the cercaria of *A. pipientis*. The tails of the two species are much alike and both have eight penetration glands. Further comparisons are impossible because Lutz' description is incomplete.

Cercaria absurda and *Cercaria bulbocauda,* both described by Miller (1927) from San Juan Island, Washington, also have enlarged and atypical tails. The tail stem of the former is sac-like and is covered with long spines, while the tail of the latter has four bulbs on the distal portions.

Azim (1935) described the successive stages in the life cycle of *Apharyngostrigea ibis,* the only other species in the genus for which the

life history is known. According to Azim's description, the cercaria of that species is similar to the cercaria of *A. pipientis* in several respects but differs in that it is apharyngeal, has no commissures in the excretory system, and has only six penetration glands.

PENETRATION EXPERIMENTS

Under experimental conditions the cercariae enter tadpoles of *R. pipiens* and continue their development. Tadpoles of both *R. pipiens* and *Hyla versicolor* collected from the field also harbored developing metacercariae of *A. pipientis*.

Cercariae were never observed to penetrate the skin of tadpoles and it is concluded that they do not enter by way of the general body surface, but penetrate the branchial or digestive epithelium. The cercariae probably enter the mouth with the respiratory water and attach to the mucosa. Experiments designed to determine the exact path of entry were abandoned because the supply of cercariae and tadpoles became too limited. Lutz (1931), who studied a species very similar to this one, also failed to observe penetration into tadpoles. Azim (1935) noted that the cercariae of *A. ibis* are pursued and devoured by tadpoles and he found the larvae in the gill clefts twelve hours later. He added that penetration of the mucous membrane of the mouth and esophagus was aided by the body spines and the secretions of the penetration glands.

DEVELOPMENT IN THE SECOND INTERMEDIATE HOST

The cercarial bodies appeared in the body cavity of experimental tadpoles within two days after exposure. The metacercariae metamorphosed in that region and encysted about the heart or on the mesenteries. The lack of a sufficient number of cercariae and suitable tadpoles made it impossible to study in detail the morphological changes which occur in the second intermediate host. Incomplete data taken from experimentally reared metacercariae are presented.

After penetration the larvae grew rapidly and became flat and leaf-like. Twenty metacercariae nine to ten days old were measured. The smallest was 1.23 by 0.47 mm while the largest was 1.60 by 0.54 mm.

During the first week of development the spines and the penetration glands were lost but the pharynx remained essentially unchanged. In three six-day larvae the pharynx measured 10 μ in diameter. The primary excretory system did not become modified during early development since in six-day larvae the flame cell pattern was the same as that of the cercariae. The formation of the reserve excretory channels is shown in Figs. 21 & 22.

The holdfast organ made its appearance in five- and six-day larvae as a small group of cells behind the ventral sucker. It developed gradually and just preceding encystment was large and prominent.

In larvae about ten days old numerous unicellular cystogenous glands began to appear in the parenchyma. These cells open on the body surface and contain a homogeneous granular secretion which is destined to form the cyst wall. As the time for encystment approached they increased greatly in size and just before encystment constituted a large proportion of the metacercarial body.

As was noted before, the pharynx was still present in six-day larvae. It disappeared gradually during subsequent development, however, so that in larvae just prior to encystment there was no pharynx. Fig. 24 traces its disappearance. Encysted metacercariae of *A. pipientis* are apharyngeal.

A comparison of the development of *D. micradenum* and *A. pipientis* reveals that the reserve excretory system originates in the cercaria of one and in the metacercaria of the other. The cercaria of *D. micradenum* has no transverse commissures while that of *A. pipientis* has two. However, two commissures form in the very young metacercariae of the former so that the reserve systems of young metacercariae of the two species are similar. Therefore, the presence or absence of transverse commissures in cercariae simply indicates differences in the rate of development of their respective excretory systems.

ENCYSTMENT

After a period of development free in the body cavity of the tadpole, the metacercariae secrete about themselves a thick, resistant cyst wall in which their metamorphosis is continued and maturity attained. The first material secreted marks the outermost boundary of the definitive cyst. The cystogenous material is extruded gradually and is laid down in concentric lamellae on the inside of this primary membrane. As the cyst wall thickens the larvae diminish in size since large quantities of secretion are poured out (Fig. 23). Near the completion of encystment, after formation of the thick outer wall, a very thin inner cyst wall is secreted which is separate from the outer one.

The time at which encystment is initiated varies considerably but it occurs relatively early in development when the metacercariae are still immature. In one experiment larvae had begun to encyst after eighteen days in the tadpole. In others, larvae were in early stages of encystment at 20, 22, and 23 days. Larvae must undergo extensive development within the cyst wall before they are mature and infective.

MATURE METACERCARIAE

(Figs. 36 & 37)

Mature, experimentally raised metacercariae correspond with almost every detail with the description of *Tetracotyle pipientis* Faust as given by

Hughes (1928a) and with cotype specimens of that species borrowed from the University of Michigan Parasitological Collection. However, there are certain differences between Hughes' description and my findings. He stated that the outer lamellar portion of the cyst wall was of host origin, while my studies on the formation of the cyst wall indicate that it is secreted by the parasite. He also stated that the fore- and hind-bodies are both covered with spines. I found no spines, but probably overlooked them since living specimens were not studied intensively.

Finally, in Hughes' description the following statement appears: "The pharynx is very small and inconspicuous, seemingly embedded in the dorsal wall of the oral sucker." As stated previously, mature cysts which I obtained have no trace of a pharynx. No pharynx is shown in Hughes' figure of *T. pipientis* and no pharynx is visible in cotype specimens of this species from the University of Michigan Parasitological Collection. Faust (1918) stated, "A small swelling within the oral sucker marks the pharynx, just behind which is the esophagus."

ADULTS

(Figs. 25–30)

Experimentally reared metacercariae were fed to a number of animals and two domestic pigeons were successfully infected. The first pigeon from which adults were obtained was fed a tadpole containing 56-day cysts. Eight days later, four worms were recovered from the small intestine. The second pigeon was fed thirty 64-day cysts and one worm was secured after eleven days. Two other pigeons were fed experimentally raised cysts. One was fed 22-day cysts, which were undoubtedly too young to be infective. The other was fed thirty 64-day cysts. Four days later the pigeon died of what appeared to be a severe digestive disorder and no worms were recovered.

The domestic pigeon is not a field host for this species and is probably refractory, since only a few worms were obtained in the successful experiments. Attempts to collect adults of *A. pipientis* from water birds of the Douglas Lake Region have been unsuccessful.

The following description of the adults of *A. pipientis* is based on the four specimens obtained from the first pigeon infected. The single specimen taken from the second pigeon is apparently abnormal and unsuitable as a source for accurate morphological data since the genital organs are abnormal and appear to have been regressing at the time of fixation although the worm was alive when fixed.

Specific Diagnosis of Adult Stage: Characteristics of the genus: Fore-body cup-shaped surrounding the large holdfast organ which fills it. Length of fore-body 0.42–0.50 mm; width 0.34–0.47 mm. Hind-body clearly set off from fore-body, usually curved dorsally, truncated at posterior end, greatest diameter at level of testes. Length of hind-body 1.23–1.45 mm; width 0.23–0.28 mm. Pair of sucking cups lateral

and posterior to oral sucker opening into the cavity of the fore-body. Lobes of holdfast organ reach the lip of the fore-body. A large, compact gland, longer than broad, composed of many groups of cells, at the base of the holdfast near the junction between the fore- and hind-bodies; dorso-ventral dimension 0.11–0.13 mm. Oral sucker on lip of fore-body, longer than broad; width 0.07–0.09 mm. No pharynx. Esophagus narrow. Digestive ceca extend to level of genital atrium. Testes tandem, lobed. Length of anterior testis 0.15–0.19 mm; width 0.13–0.14 mm. Length of posterior testis 0.15–0.20 mm; width 0.13–0.14 mm. Ovary ovoid, anterior to testes; length 0.12–0.16 mm; width 0.09–0.10 mm. Vitellaria predominantly ventral throughout hind-body, voluminous between ovary and large gland at the base of the holdfast. Vitellaria sparse in the fore-body but occur in dorsal and lateral walls of the fore-body and in the base of the holdfast organ. Genital atrium deep, surrounded by muscular wall.

Habitat: Small intestine.

Host: Domestic pigeon (experimental).

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 36700.

Male Reproductive System

The anterior testis, testis I, lies behind the ovary and always consists of several large lobes. The posterior testis, testis II, has almost the same size and form as testis I and is separated from it by the female genital complex. The vas efferens of testis I arises between the lobes on the anterior surface, curves dorsally and enters the region behind the ovary. The vas efferens of testis II arises from the anterior surface, bends ventrally, and then proceeds along the ventro-lateral margin of testis I to the region between the latter and the ovary. The vasa efferentia make several loops in this region before uniting beneath the ovary to form the vas deferens. The latter runs posteriorly beside the descending uterus. Behind testis II it increases in diameter to form the seminal vesicle which is coiled. The seminal vesicle narrows before opening into the terminal genital atrium.

Female Reproductive System

The oviduct arises from the postero-dorsal surface of the spherical to ovoid ovary. It makes a loop behind the ovary and then, at the anterior margin of testis I, communicates with Laurer's canal which is short and narrow and opens dorsally. The oviduct continues posteriorly in a dorsal cleft in testis I. Immediately after receiving the common duct from the large vitelline reservoir in the intertesticular region, it enters Mehlis' gland and becomes the ootype. The latter is continued as the ascending uterus which makes a loop in the region of Mehlis' gland and then proceeds to the anterior portion of hind-body. Here it turns and becomes the descending uterus which extends along the mid-line, ventral to the other organs, and opens in the genital atrium.

DISCUSSION

Szidat (1929a, p. 665) suggested that apharyngeal strigeid cercariae probably constitute a stage in the life cycle of species in the genus

Apharyngostrigea. Lutz (1931, p. 342) pointed out, however, that apharyngeal adults might develop from pharyngeal cercariae because there is such an extensive metamorphosis in the second intermediate host. Azim's (1935) description of the life cycle of *A. ibis* supports Szidat's view since the cercaria of that species is apharyngeal. Azim stated "Das Fehlen eines Pharynx in allen Stadien der Entwicklung des Trematoden kennzeichnet ihn als zur Gattung *Apharyngostrigea* Ciurea (1927) gehörig." My findings, on the other hand, bear out Lutz' contention and indicate that members of this genus may have either apharyngeal or pharyngeal cercariae.

The only other known life history in the genus is that of *A. ibis* (Azim, 1935). According to Azim, the cercariae of this species are apharyngeal and develop in *Planorbis boissyi* Pot. and Mich., *Bullinus dybowskyi* Fisch., and *Pyrgophysa forskali* Ehrenb. These cercariae penetrate tadpoles of *Bufo vulgaris* Laur. and develop into encysted tetracotyles which become adults when eaten by the ibis, *Ardeola ibis ibis* (L.).

Lutz (1931) described cercariae from *Planorbis immunis* Lutz which developed to encysted tetracotyles after penetrating *Poecilia vivipara* and tadpoles of *Leptodactylus ocellatus.* After feeding these cysts, he obtained adults of *Hysteromorpha triloba* from *Nyctanassa violacea* and *Herodias egretta.* But since the experiments of Lutz were not well controlled, the results are open to question. *Hysteromorpha triloba* is a pharyngeal species belonging to the DIPLOSTOMIDAE while my studies with cercariae and metacercariae very similar to those figured by Lutz (Lutz, 1931, Figs. 1, 2, 15) indicate that larvae of that type are stages in the life cycle of *Apharyngostrigea pipientis* which is apharyngeal and belongs in the STRIGEIDAE. Moreover, Ciurea's (1930) studies on *H. triloba* in Roumania indicate that this species develops from metacercariae different from those figured by Lutz.

TAXONOMIC RELATIONS

Dubois (1938) included eight species in the genus *Apharyngostrigea* and Olsen (in press) has since added *A. bilobata.* The ovoid ovary, weakly lobed testes, narrow hind-body, broad fore-body, and large holdfast gland, taken together, distinguish *A. pipientis* from all the previously described species in the genus.

SUMMARY

The life histories of *Diplostomum micradenum* (Cort and Brackett) and *Apharyngostrigea pipientis* (Faust) are described.

The cercariae of *D. micradenum* (*C. micradena* Cort and Brackett) penetrate tadpoles and develop in the central nervous system to diplostomula with lateral sucking cups.

The metacercariae of *D. micradenum* develop into adults in the small intestine of the domestic pigeon.

The cercariae of *A. pipientis* penetrate tadpoles and develop into encysted tetracotyles (*Tetracotyle pipientis* Faust). The pharynx is lost during the metamorphosis of the metacercariae.

Metacercariae of *A. pipientis* develop into adults in the small intestine of the domestic pigeon.

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Explanation of Plates

Abbreviations

cvd	common vitelline duct	os	oral sucker
ejd	ejaculatory duct	ov	ovary
ep	excretory pore	sv	seminal vesicle
ex sp	excretory space	t 1	anterior testis
hf	holdfast organ	t 2	posterior testis
hf gl	accessory gland of the holdfast	ut	uterus
int	intestinal ceca	v	vitellaria
lc	Laurer's canal	vd	vas deferens
lip	lip of the forebody	ve t1	vas efferens of anterior testis
lum hf	cavity of the holdfast organ	ve t2	vas efferens of posterior testis
nc	nerve commissure	vit	vitelline duct
od	oviduct	v res	vitelline reservoir
oot	ootype	vs	ventral sucker

Plate I

STAGES IN THE LIFE CYCLE OF *DIPLOSTOMUM MICRADENUM*

The Metacercaria

Figs. 1–5. Immature metacercariae drawn to approximately the same scale from living specimens. The formation of the reserve excretory channels and transformation of the oral sucker are shown.

Fig. 1. Three-day larva.

Fig. 2. Four-day larva.

Fig. 3. Five-day larva.

Fig. 4. Six-day larva. The complete primary excretory system is shown. Note the remnant of the tubule which entered the tail of the cercaria.

Fig. 5. Eight-day larva.

Fig. 6. Eight-day larva which had developed more rapidly than the specimen in Fig. 5.

Fig. 7. Drawing of mature metacercaria from living specimens. Only the main channels of the reserve excretory system are shown on one side. The terminal portions of the tubules with their concretions are not shown. Dorsal aspect.

Fig. 8. Camera lucida drawings of four metacercariae to illustrate characteristic shapes assumed by living larvae.

The Adult

Fig. 9. Reconstruction from cross sections showing the union of the vasa efferentia to form the vas deferens at the level of the anterior margin of testis I. Anterior aspect. Dorsal surface on the left.

Fig. 10. Reconstruction of portions of the female genital system from cross sections. Anterior aspect. Dorsal surface on the left.

Fig. 11. Reconstruction of the female genital system. Lateral aspect.

Fig. 12. Reconstruction of male genital system. Ventral aspect.

Fig. 13. Ventral aspect of adult showing the lateral sucking cups, the digestive system, and the extent of the vitellaria. The limits of the vitellaria are indicated by the broken line.

STAGES IN THE LIFE CYCLE OF *APHARYNGOSTRIGEA PIPIENTIS*

The Cercaria

Fig. 14. Drawing of a cercaria under slight cover glass pressure showing contracted tail and body partially enveloped by it.

Fig. 15. Ventral aspect of tail showing circular muscles, longitudinal muscle bands, a row of cells on median ventral line, hair-like structures scattered on tail-stem.

Fig. 16. Optical section of tail showing the excretory tubule and the large cells associated with it.

Fig. 17. Lateral aspect of tail showing two longitudinal groups of contractile fibers and their insertions in the furca.

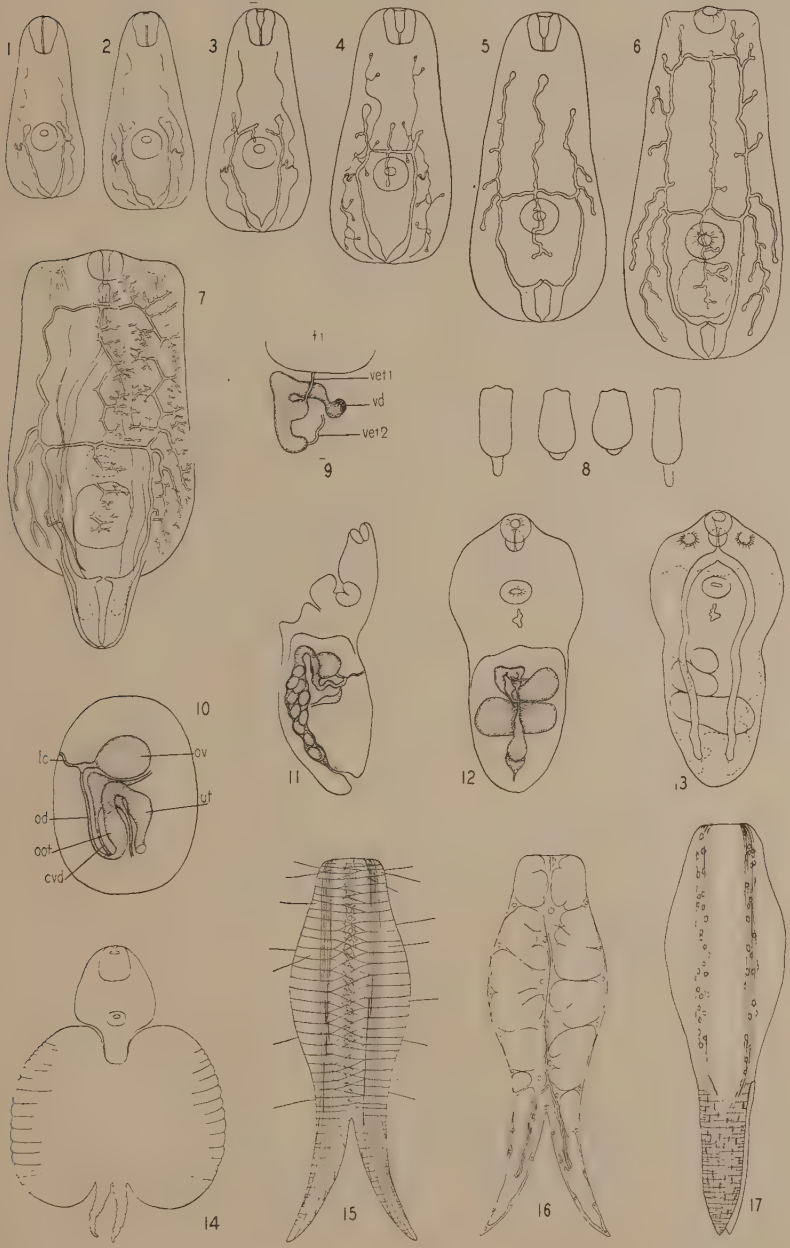
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Plate II

STAGES IN THE LIFE CYCLE OF *APHARYNGOSTRIGEA PIPIENTIS*

The Cercaria

Fig. 18. The body of the cercaria. Ventral aspect. Primary excretory system and spination are shown only on one side.

Fig. 19. Anterior end of daughter sporocyst drawn from a living specimen, showing birth canal and mobile anterior end. The sporocyst was 1.5 mm long.

The Metacercaria

Fig. 20. Drawing of a metacercaria just prior to encystment. The fundaments of the holdfast and its associated gland are shown behind the ventral sucker. Ehrlich's hematoxylin. Ventral aspect.

Fig. 21. Drawing of a living six-day metacercaria in ventral aspect. The glands, portions of their ducts, and the pharynx are still present. The primary excretory system is shown on the right. The reserve excretory system is shown completely only on the left.

Fig. 22. Drawing of a living five-day metacercaria in ventral aspect. Development of the reserve excretory system is farther advanced than in an older larva shown in Fig. 21, probably because environmental temperatures were higher during its period of development.

Fig. 23. Three sketches drawn to the same scale, showing formation of the thick external wall of the metacercaria. The stippled area indicates the space occupied by the metacercaria.

a. Early stage in encystment. The wall is thin and the parasite large.

b. The wall has thickened and the parasite is smaller.

c. Mature cyst. The loss of cystogenous material has reduced the size of the worm considerably.

Fig. 24. Drawings taken from sections of metacercariae to illustrate the disappearance of the pharynx as maturity is reached.

a. Oral sucker and pharynx of a metacercaria about six days after penetration. The worm was 5 mm long and the pharynx was 10 μ in diameter.

b. Oral sucker and pharynx of a nine-day metacercaria. Note the gland ducts.

c. Oral sucker and esophagus of a 14-day metacercaria. The esophagus is large but there is no pharynx.

d. Sagittal section of oral sucker and esophagus of a 21-day metacercaria (encysted). The pharynx has completely disappeared.

e. Section of esophagus and portion of oral sucker of a 63-day metacercaria.

f. Sagittal section of 34-day cyst showing oral sucker and esophagus with no trace of a pharynx.

The Adult

Fig. 25. Drawing of a sagittal section.

Fig. 26. Reconstruction of the female genital complex between the testes. Anterior aspect. Dorsal surface up.

Fig. 27. Reconstruction of vasa efferentia and their union to form the vas deferens. Anterior aspect.

Fig. 28. Reconstruction of female genital system.

Fig. 29. Reconstruction of male genital system.

Fig. 30. Camera lucida drawings of two fixed specimens.

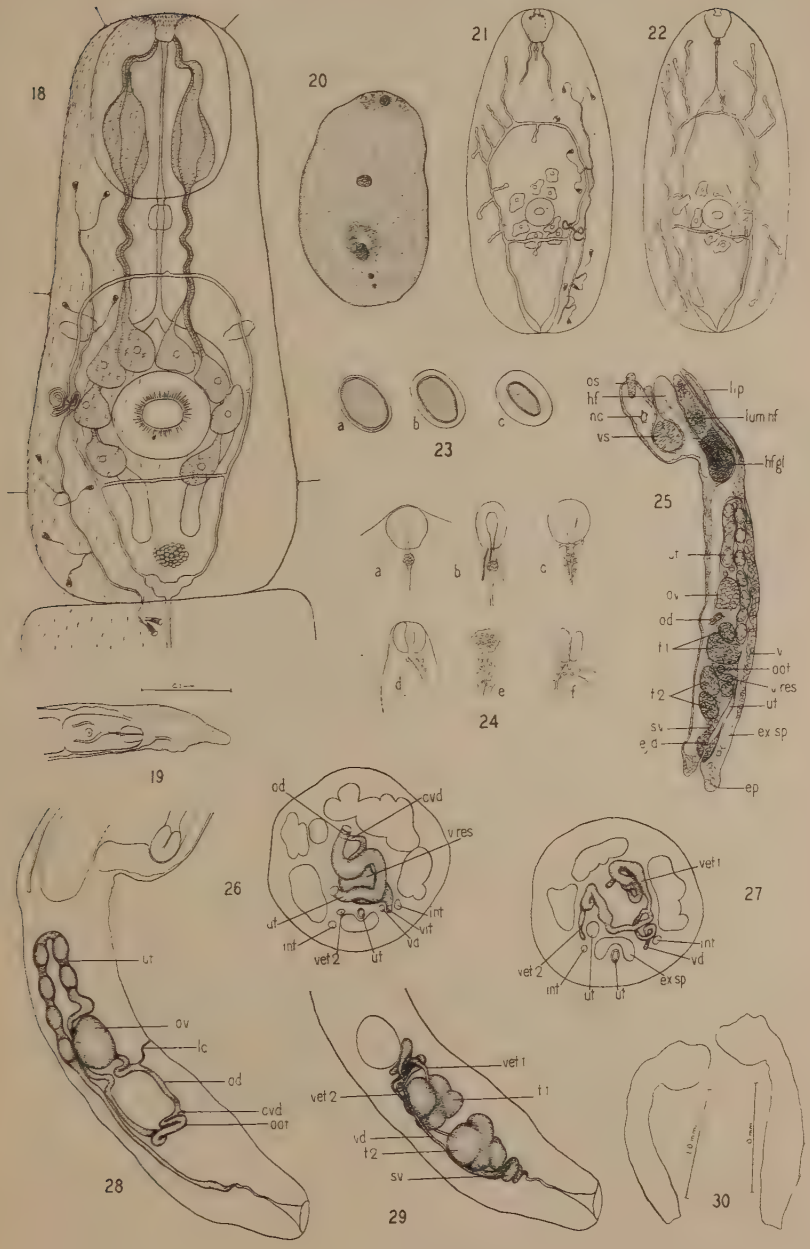
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Plate III

STAGES IN THE LIFE CYCLE OF *APHARYNGOSTRIGEA PIPIENTIS*

Fig. 31. Photomicrograph of uncleared, fixed cercariae of *A. pipientis*.

Fig. 32. Photomicrograph of immature unstained metacercariae of *A. pipientis* cleared in glycerine. The largest were more than 1 mm long and ten days old. The smallest were about three to five days old.

Fig. 33. Whole mount of mature metacercaria of *D. micradenum*. Note the lateral cups, suckers, holdfast and elongate hind-body. Photomicrograph. Ehrlich's hematoxylin.

Fig. 34. Sagittal section through the brain of an experimentally infected tadpole of *Rana pipiens* showing metacercariae of *D. micradenum* under the meninges. Photomicrograph. Mallory's triple stain.

Fig. 35. Sagittal section through the spinal cord of an experimentally infected tadpole of *Rana pipiens* showing metacercariae of *D. micradenum* within the spinal canal and under the meninges dorsal to the cord. Photomicrograph. Mallory's triple stain.

Fig. 36. Photomicrograph of two 64-day excysted metacercariae. Fixed and stained in paracarmine.

Fig. 37. Photomicrograph of living, encysted metacercariae 64 days old.

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THE BIOLOGY AND ECONOMIC IMPORTANCE OF A NEMATODE PARASITIC IN INSECTS[1]

R. W. Glaser, E. E. McCoy and H. B. Girth

In 1932 (1) a circular appeared which dealt with the biology and culture of *Neoaplectana glaseri* Steiner (2), a nematode parasite of the Japanese beetle (*Popillia japonica*). During the past eight years work with this parasite has continuously progressed in a number of directions. Consequently, much new information has accumulated, some minor mistakes have been corrected, and certain details elaborated. This article brings the subject up-to-date.

In the above mentioned publication, some of the more important work which dealt with parasitism of insects by nematodes was reviewed. Bovien in 1937 (3) dealt more fully with the literature on the subject. Insofar as the writers are aware nothing has recently appeared that has any direct bearing on the present account, and therefore another treatment of the work of others would seem superfluous. Bovien's original investigations (1937), however, with *Neoaplectana bibionis* and *N. affinis* had a profound effect on the further course of our economic endeavors, as will appear later. Bovien himself fully realized the significance of his observations by stating: "The most important result of my investigations was the finding of a morphologically and biologically well defined 'Dauerlarve,' a stage which is not obligatory, but only found under certain external conditions. This stage has proved to be the infective stage as well."

LIFE HISTORY

The life history of *Neoaplectana glaseri* on the various culture media devised for propagating the parasite is similar to the life history in nature. The following description applies in a general way to the development of the organism when being cultured on artificial media as well as when it is parasitic in any of its insect hosts.

In common with many nematodes parasitic in or associated with insects, *N. glaseri* has a free-living phase, the normal termination of which is characterized by the retention of the cuticle of the second-stage larva. This retained cuticle forms a loose coat about the enclosed third-stage larva, which is then said to be "ensheathed." As the third cuticular covering is formed, the internal structure of the nematode undergoes certain changes which, while minor in appearance, seem to be of consider-

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[1] Conducted cooperatively by The Rockefeller Institute for Medical Research, Princeton, N. J., the New Jersey Department of Agriculture, and the United States Department of Agriculture.

able biologic significance. In this species the sheath is not very tenacious, and, due to the rather unusual degree of activity of the nematode, is usually lost soon after the larva assumes a free-living existence in the soil. When the sheath is lost the larva is spoken of as an "exsheathed" larva. Bovien (3) has recently published an account of the association of insects and nematodes in which he refers to this larval form as the "Dauer" stage, a term adopted from Fuchs (4). English usage requires the use of the terms "invasive" or "infective" stage, and in this paper the term "infective stage" will be used to denote the free-living stage, irrespective of the presence or absence of the sheath.

The infective stage larvae (Fig. 3) vary in length from 0.85 mm to 1.45 mm, and the ratio of length to width is approximately 29:1. The larvae are dirty white in color, but since they are transparent only from the oesophageal bulb forward and in the extreme caudal region, they appear dark when viewed under the microscope by transmitted light. When warm, the larvae are very active if they are mechanically stimulated. However, when they are in a perfectly quiet environment, periods of quiescence and activity alternate. Development of the nematodes from the infective stage to the pre-adult or fourth stage is dependent upon access to an acceptable food supply, which in the soil, occurs only in the form of the various host insects. A number of culture media have been devised which permit the larvae to develop under artificial conditions. If the sheath is still present when the nematode finds a food supply, it is soon lost due to the constant pressure of the nematode against the forward end. The exsheathed larva develops to the pre-adult stage.

The pre-adult forms are quite slender, semi-transparent, and active. Pre-adult males (Fig. 5) measure about 1.0 mm, and pre-adult females (Fig. 4) from 1.4 to 1.6 mm in length. The pre-adult forms develop to the adult male and female nematodes, which latter are the fifth stage of development, requiring four ecdyses for the complete development from the first-stage larvae.

The adult male (Fig. 6) is rather slender and is semi-transparent, averaging about 1.4 mm in length. Males are easily distinguished from females by their smaller size, greater activity, and the characteristically curved posterior end. The brownish colored spicules are conspicuous. The females (Figs. 7 and 8) are almost transparent, rather plump, and usually measure about 4.7 mm in length. The protruding vulva, approximately median, is very conspicuous in the females. The females vary greatly in size. In parasitized Japanese beetle larvae, mature females only 2.5 mm in length sometimes occur, while others have been found measuring as large as 9 mm in length. The large or "giant" females have been described by the authors (5).

After becoming mature, the adult nematodes copulate. The females

must be fertilized by the males in order to produce offspring. The females are ovoviviparous, and young larvae as well as ova in various stages of cleavage may be seen simultaneously in the uterus, which occupies a large proportion of the body cavity. Larvae are born one or two days after the female reaches the adult stage. Steiner (2) states that "it is probable that most of the larvae hatch only after the mother is dead." This is true in some cases, but far more frequently the mother is alive and plays a decided role in the birth of her young. On hatching from the eggs, the young larvae do not seem to be perfectly developed, and are sensitive to slight changes in the environment. While still within the uterus they wander about for a considerable period, growing somewhat, and occasionally trying to escape at the vulva. Finally a young larva makes persistent attempts, and suddenly the vulvar muscles relax and the larva is expelled with great rapidity. Another birth may not occur for an hour or two. The majority, and sometimes all, of the offspring are born in this manner. Should the mother die before all of the larvae are born, the young remain in the nearly depleted uterus of the dead mother for several days, and may reach the second larval stage before finally being liberated through their constant efforts. The females produce about 15 young each, although the "giant" individuals may produce as many as 1400 young.

In at least one closely related nematode, it has been observed that the eggs are frequently deposited and hatch outside the mother. This has never been observed in *N. glaseri*.

The first-stage larvae (Fig. 1) are small, slender, and wholly transparent, measuring from 0.35 to 0.50 mm in length. They grow rapidly and within two days transform to the second larval stage.

Second-stage larvae (Fig. 2) are active, slender, and quite opaque except in the oesophageal and caudal regions. They appear black under a microscope with transmitted light, but are actually creamy white in color. They measure from 0.85 to 1.45 mm in length, so that the length is identical with that of the infective stage. The length-width ratio approximates 23 : 1, so that the form is somewhat more plump than is the infective stage.

At this point a rather peculiar phenomenon occurs. If the second-stage larva is in an environment favorable for further development, the pre-adult stage follows it directly. This occurs in the insect hosts as well as on the artificial media. In this case the third larval stage (i.e., the infective stage) is suppressed, and the nematode completes its life history with three ecdyses. However, if the second-stage larva is in an environment not suited for further development, such as an insect cadaver already exhausted of available food, the larva ceases to grow, empties the alimentary tract, and forms a new cuticle. Simultaneously the older

cuticle becomes loosened, first being noticeably separated at the head, and in this way the sheath of the infective form develops (Fig. 3). The diameter of the second-stage nematode decreases by about 22 per cent during these changes, while the length remains practically constant. The sheath therefore is loose laterally, but initially fits closely lengthwise. The significance of these facts was not fully appreciated until Bovien's work appeared, but they are of immense practical importance, as will be seen later.

Development of the nematode is rapid. Under optimum conditions the life cycle may be completed within 5 to 7 days, depending upon whether or not the third stage occurs.

RELATION WITH INSECT HOSTS

The infective stage nematodes live in the soil, where they are rather active during periods when the soil is moist and warm. They are capable of a sustained existence of at least one and one-half years in the absence of host insects. It is probable that most of the larvae live free in the soil during the winter, though some may be in host insects during this period. During the warmer periods of the year the nematodes actively parasitize host insects present in the soil, but such attack is very limited at a soil temperature of 10° C, and does not become significant until the temperature reaches 18° C. The larvae enter the hosts through the mouth with the food.

Within the host, the nematodes develop rapidly, and the second-stage larvae of the new generation may appear within five days from the time the infective stage entered. In most cases the first generation of second-stage larvae to appear in the host develop directly to the pre-adult stage, since an abundance of food is present, and the ensheathed (or infective) form does not appear until later, when the food supply is exhausted. In most cases the host insect dies by the time the first generation of second-stage larvae has developed. Further nematode development continues in the dead host, and under these conditions the nematode must be considered a saprozoic organism rather than a parasite. This leads one to the conclusion that *N. glaseri* is a species in transition from the free-living, saprozoic mode of existence to a parasitic one. As many as three generations may occur within one host, two of these appearing after the host's death. In this respect this species exhibits a behavior greatly at variance with that of the characteristic nematodes of vertebrates.

Occasionally an insect host becomes parasitized very lightly, so that only one nematode becomes successfully established. This individual may be of either sex, and while it frequently (if not always) causes the death of the host, there is no reproduction. Where extremely dense parasite populations exist, multiple parasitism of the host may occur to such

an extent that the nematodes become over-crowded before completing the life cycle, in which case they die. This latter occurrence is not frequent in areas where the parasite has been established for some time, but is found in areas immediately following artificial introduction, due to local concentration of the parasites.

After death, Japanese beetle larvae killed by the nematode are most often of a brownish color. Color of the cadaver, while suggestive, is no criterion of parasitism. Proof of parasitism can be established only by a microscopical examination. The parasitized cadavers are very flaccid, since as the nematodes feed, all of the body tissues are attacked and broken down. Finally, all that remains of the host grub is a skin and head capsule, filled with a thin fluid swarming with the larval nematodes. When this point is reached, the nematodes cease development at the second stage and ensheath. The weakened integument of the host larva then breaks and the ensheathed, or infective, larvae are liberated in the soil. As many as 2400 infective stage larvae have been recovered from one Japanese beetle grub; however, the average successful case of parasitism probably does not produce as many as 1500 new parasites.

Neoaplectana glaseri does not confine its attack to the larvae of the Japanese beetle. Adult Japanese beetles are readily parasitized when they are present in the soil. Suitable conditions for such infection prevail (a) when the female beetles enter the soil for oviposition and (b) during the period between the completion of metamorphosis and the escape of the adult from the pupal cell. These parasitized adults are sometimes capable of flying for a short period before they are killed by the parasite. It is probable that any extensive natural infestation of a new area must be through the agency of these parasitized adults. Under field conditions it has been demonstrated that the parasitized adults are capable of initiating new infection foci.

It has recently been found that this nematode attacks a number of insects in addition to the Japanese beetle. Parasitism of the larvae of *Anomala orientalis* occurs with a facility at least equal to that observed in the Japanese beetle grubs. In addition, the larvae of several species of *Phyllophaga, Autoserica castanea, Macrodactylus subspinosus, Ochrosidia villosa, Cotalpa* (sp.), *Cotinis nitida,* and *Xyloryctes satyrus* are all infected with more or less ease. The larva of the white-fringed beetle, *Pantomorus leucoloma,* is likewise attacked.[2] In field plots the pupae of elaterid beetles and noctuid larvae have been recovered, in each case with the insects dead and the nematodes apparently thriving. Some insect larvae, not normally soil inhabiting, may be infected by confining them in nematode infested soil; this occurs with larvae of the European corn borer, *Pyrausta nubilalis.*

[2] These tests as to *P. leucoloma* were performed by Mr. H. C. Young of the U. S. Division of Cereal and Forage Insect Investigations at Florala, Alabama.

In all of the hosts cited above, nematode development proceeds in a normal manner. The degree of resistance to parasitism is variable, possibly the most susceptible insect being *Anomala orientalis,* and the least susceptible being *Ochrosidia villosa.* It is apparent that *Neoaplectana glaseri* is a more or less general parasite of insects, and that there is justification for field investigations of its potentialities in the control of a number of insects in addition to the Japanese beetle.

ARTIFICIAL CULTURE OF *Neoaplectana glaseri*

Soon after the discovery of the nematode it was thought that if the parasite could be artificially cultured, much that was obscure about the biology could be determined, and that a method for the biological control of the Japanese beetle might be evolved. In 1931 the first such culture method was developed (6), and in 1936 (7) and 1938 (8) two additional methods were described.

Agar Cultures

Culture of the nematodes was first successfully accomplished using dextrose-veal infusion agar plates, on which a heavy growth of baker's yeast was grown before planting the nematodes. This method is still used for maintaining a breeding stock of nematodes because of its simplicity. The agar medium is not well suited to the culture of the large number of nematodes required for field distribution.

The agar medium is prepared according to a standard bacteriological formula, using the heated infusion from 500 gm of fresh, lean, ground veal per 1000 ml of finished medium, and incorporating 1.0 per cent peptone, 0.5 per cent sodium chloride, 2.0 per cent dextrose, and 2.0 per cent agar. The medium is adjusted to a reaction of pH 7.2. After autoclaving the medium in plugged flasks, it may be stored until required for culture use.

The agar is melted and poured into sterile Petri dishes so that a layer about 3 mm deep is formed in each dish. When the plates have cooled, the agar surface is flooded with a suspension of pure baker's yeast in sterile water. The plates are incubated at 27° C for at least 24 hours, during which time a heavy culture of yeast will be obtained on the agar surface. The plates are then ready to be inoculated with the nematodes.

The nematodes may come from an infected insect or from a previous culture. In the former case the insect is triturated in a small volume of water before being placed on the culture plate. Since it frequently happens that various species of nematodes are associated in insect cadavers, it is necessary to purify the cultures in order to obtain a persistent culture of *Neoaplectana.* Differentiation of the nematode species is most easily made when the gravid females of *Neoaplectana* appear on the

initial cultures, which may be 3 or 4 days after the inoculation. The gravid females are removed individually from the surface of the initial culture by means of a fine insect pin having the tip bent at an angle of about 90°, and mounted in a wooden or glass handle. The females removed from the initial culture should first be transferred to a few drops of water in a depression slide, and later individually transferred to a fresh agar culture. This work should be done under a binocular microscope with a magnification of about 30, in order to be certain that no larval forms, or eggs, of other species of nematodes are transferred to the new cultures. A dozen females are sufficient to inoculate one 50 mm plate.

Heavier yields and more consistent cultures result if finely ground bovine "ovarian substance" is sprinkled over the culture surface just after the nematodes are planted. About 0.001 gm of the material is used per square centimeter of culture area. The nematodes feed upon this in addition to the yeast cells and other culture nutrients. Nematodes cultured without the "ovarian substance" die after six or seven transfers, whereas those to which it is added may persist for as long as thirty transfers or longer.

Nematode development follows the course described in the life history section. It is customary to wait until the second group of second-stage larvae appear before making a transfer from the initial purified cultures, this being done because of the small number of nematodes used as inoculum. It should be noted that after the first persistent culture, all further transfers are made with a heavy nematode inoculum so that the maximum yield is obtained in one generation.

Nematode cultures are incubated at 21–24° C, and the initial cultures require 7 to 10 days for the second group of second-stage larvae to appear. The mass on the culture surface should be maintained at such a fluidity that it will slowly flow over the surface when the plate is tilted. Distilled water is added as needed to maintain this condition.

The nematodes are removed from the cultures when the second-stage form appears in greatest numbers. Sterile water is run over the culture surface and the mass of nematodes and culture debris worked into a suspension, which is then poured into a large test tube. The worms are washed by repeated sedimentation through the water column, each time drawing off the supernatant fluid after the worms settle and replacing with fresh water. Sedimentation cannot completely remove the culture debris, and for some purposes it is necessary to permit the nematodes to migrate through Japanese lens paper, which holds back most of the larger particles. The lens paper, in single or double thickness, may be conveniently supported in a small flat-bottomed sieve, with the bottom in contact with water in a beaker, funnel, or other vessel. The nema-

todes wriggle through the interstices of the paper and sink in the water. After one or two final sedimentations, which remove the remaining fine particles, the nematodes are exceptionally clean.

The washed nematodes may then be transferred to fresh cultures. The rate of inoculation should be such that one generation of growth on the fresh culture will produce the maximum possible yield. The inoculum should be from 20 to 40 nematodes per sq cm of culture area. The density of population at the end of the culture period may vary from 400 to 800 individuals per sq cm of culture area, corresponding to a tenfold to twentyfold increase. Distinct cultural characteristics occur in nematodes from different insect cadavers, and it is well to begin with a number of these "strains," later selecting those best adapted to the work. There is a slow decline in fecundity of the cultured nematodes, some "strains" dying out after 5 or 6 transfers, while others continue to yield good cultures after 20 or more transfers. In general, it is better to discard the stock after 10 or 12 transfers and start with material freshly isolated from infected insects in order to keep the productivity high. Failing cultures can be reinvigorated by passage of the nematodes through three or four Japanese beetle grubs in succession.

Under the conditions outlined above, a generation of nematodes will be produced within 7 days, and transfers are customarily made at weekly intervals. It should be noted that the nematodes do not ensheath when transferred in this manner. Old cultures, in which the nematodes have reached stability at the second stage, will show some ensheathed larvae 3 or 4 days after growth ceases. However, if it is desired to obtain the ensheathed form from agar cultures, it is preferable to remove the washed second-stage larvae to water and keep them under conditions to be described later.

Potato Culture Medium

The potato culture medium (7) was developed after it became evident that the agar culture technique was inadequate to supply the number of nematodes required for large-scale field experimentation. The medium is no longer used, since culture on a veal pulp medium has been found much more satisfactory. The potato medium consisted of a mixture of ground raw Irish and sweet potatoes, which was fermented by baker's yeast before placing the nematodes on the cultures. Potato cultures produced about 2000 nematodes per sq cm of area, with occasional cultures yielding as high as 8000 nematodes per sq cm.

Veal Pulp Medium

Attempts to use meat as a culture medium were made early in the search for a suitable culture procedure, but did not prove successful at that time. The difficulties which arose in the use of the potato medium

stimulated a reinvestigation of the possibilities of meat. These later tests indicated that the rapid bacterial decomposition of the meat was probably responsible for previous failures. The addition of sodium methyl-p-hydroxybenzoate as a preservative of the ground meat resulted in a marked improvement in the cultures. Extraction of a portion of the water-soluble constituents of the meat produced a further improvement. Various kinds of meat were tried, but lean veal was found best adapted for the purpose and has been in routine use since 1936. A detailed description of the veal culture procedures has been published (8) and the method will be presented here only in outline.

The veal used in the preparation of the medium must be reasonably free of fat, and ground three times through a food chopper. The ground veal is weighed into a clean vessel, mixed with twice its weight of distilled water, and the infusion kept in the icebox for 18 to 48 hours. The infusion is then poured on a clean flannel cloth, drained, and squeezed as dry as possible by hand, or in a suitable press. About a 20 per cent loss of weight should occur during the infusion and squeezing. The expressed juice may be used for making the nutrient agar described above.

The pulp is now ready for the addition of the preservative, which is introduced in sufficient water to restore the final mixture to the initial veal weight. For all cultures intended for further culture work the preservative used is the sodium derivative of methyl-p-hydroxybenzoate, enough being added to make the final concentration of the methyl ester 0.20 per cent by weight.[3]

The mass is then thoroughly mixed to distribute the preservative, after which the medium is ready to be placed in the culture dishes. When the nematodes are to be ensheathed prior to field distribution, it has been found preferable to prepare the medium with a concentration of 0.05 per cent hydroxybenzoate plus 0.06 per cent of formaldehyde;[4] only

[3] Preparation of preservative solution: All calculations are made on a weight-percentage basis, and for the active ingredient only.

Methyl-p-hydroxybenzoate is insoluble in water. The sodium derivative of this ester is water soluble, and is prepared as follows: In a liter flask, place 250 ml of 95 per cent ethyl alcohol, add 100 gm of the ester and about 200 ml of distilled water. Add a few drops of phenolphthalein indicator solution, and then add 5 N sodium hydroxide (200 gm NaOH per liter of solution) until all the ester is dissolved and the solution faintly pink. This operation should be done slowly, with constant shaking. Distilled water is then added to make the final solution to 1000 ml and this stock solution preserved in a rubber-stoppered bottle. This is a 10 per cent weight-volume solution, 1.0 ml containing 0.1 gm of the active principle. 2.0 ml of solution per 100 gm of veal gives a 0.2 per cent concentration. The solution keeps indefinitely under room conditions.

[4] Formaldehyde: Dilute one volume of USP grade formaldehyde (commercial formalin) with three volumes of water. This gives an approximate 10 per cent solution, and calculations are made on the assumption that 1.0 ml contains 0.1 gm formaldehyde gas. The solution must be kept cool, and made up frequently in small volumes. 0.6 ml per 100 gm veal gives a 0.06 per cent concentration.

the last transfer should be made to this medium. This change in preservatives has been found to give better results because the resulting nematodes appear more nearly isotonic to water, and therefore ensheath with a minimum of difficulty.

The prepared veal medium is spread in a uniform layer in the culture dish or tray so that the layer is about 4 mm in thickness and the surface as smooth as possible. The cover of the culture dish must not fit too tightly. The cultures are now ready for the nematode inoculum. The nematodes may come from agar cultures, or from previous veal cultures. It is not customary to use nematodes obtained directly from infected insects because of the difficulty encountered in purifying such cultures from other species of nematodes frequently found in the insects.

When nematodes grown on agar are transferred to the veal medium, the procedure of washing and migrating through lens paper is followed (see agar culture technique). Finally, the nematodes are sedimented into the smallest possible volume, and applied drop-wise to the culture surface using a pipette. Nematodes direct from agar cultures are always started on veal medium containing 0.2 per cent of the hydroxybenzoate preservative.

Nematodes from matured veal cultures are most frequently used as inoculum. In such cultures the nematodes are practically all in the second larval stage. The nematodes will not, in general, be isotonic to water, and must be washed in an isotonic sodium chloride solution. The isotonic reaction of the nematodes is determined by placing samples in salt solutions varying by 0.25 per cent until an approximately isotonic solution is found. Usually, a 1.0 to 2.0 per cent solution is required. The nematodes are washed from the surface of the matured culture using the indicated isotonic solution, and cleaned by repeated sedimentation through fresh solution. After four or five sedimentations the supernatant fluid is usually clear, indicating that the nematodes have been sufficiently washed. The washed nematodes are concentrated to a minimum volume in a small tube, and applied drop-wise to the new culture surface, using a pipette having a 3 mm orifice. The amount of inoculum used is important, as it is desirable to have the maximum possible yield after one generation, since it is only under this condition that the cultures will reach stability at the second larval stage. The approximate rate of inoculation is 600 nematodes per sq cm of culture surface, which corresponds to about 6 ml per 1000 sq cm, or one drop per 16 sq cm of area, when using the dense nematode suspension and large orifice pipette.

After inoculation the cultures are incubated for development at a temperature of 21° C (± 2°). By the 7th day the number of second-stage larvae usually reaches the maximum and the nematodes are ready to be transferred to new cultures, or removed and ensheathed.

The yield of the veal cultures ranges from 9,000 to 12,000 nematodes per sq cm of culture area. As many as 18 successive transfers on the medium have been made, with an approximate fifteenfold increase in numbers at each transfer. However, it is customary to practice continuous transfer on the medium for only about ten successive transfers. Older cultures should be replaced by beginning new cultures with agar-reared nematodes, as this practice maintains a higher average yield and more uniform development of the cultures.

ENSHEATHING OF THE CULTURED NEMATODES

The nematodes present on matured cultures are preponderantly second-stage individuals. A high mortality occurs if such nematodes are introduced directly into the soil, because the conditions in soil are not favorable for the survival of the second-stage nematodes, and most of them are unable to ensheath. It is preferable to have the nematodes ensheath before the field distribution is made. Using the methods developed, about 95 per cent of the cultured nematodes successfully ensheath, whereas only about 15 per cent will ensheath if the cultured parasites are introduced directly into the soil. It has been remarked that the nematodes normally existing in soil are in the ensheathed larval stage.

Ensheathing occurs when the second-stage nematodes are removed from culture and placed in an environment favorable for survival, but not for growth to the pre-adult stage. This condition is fulfilled by removing the nematodes from the cultures, washing, and keeping them in an isotonic salt solution until they have ensheathed. The nematodes must not be too crowded, and the solution must be replaced whenever it becomes dirty. The temperature must be high enough to permit the biological activity attendant upon ensheathing, the optimum being about 24° C.

Prior to ensheathing, the second-stage nematodes, which have been feeding on the cultures, void the contents of their alimentary tracts. This fouls the solution so that the nematodes should be washed and replaced in fresh solution about 48 hours after removal from the cultures. Ensheathing is completed within 4 days at the optimum temperature, while lower temperatures require a longer time.

Small numbers of nematodes may be ensheathed by placing them in a dish containing a layer of isotonic solution about 3 mm deep. Much larger numbers may be ensheathed by keeping the solution agitated so that the nematodes remain in suspension. The simplest procedure is to place the suspension in bottles, and arrange the bottles so that a thorough agitation is secured by bubbling compressed air through the liquid. The bottles are placed in a rack which holds them in a position tilted 45°

from the vertical. With the bottle in this position the nematodes tend to gravitate to the lowest corner, rather than over the entire bottom. Compressed air, flowing through a glass tube extending to this low corner, effectively prevents the local concentration of the nematodes at any point. After the nematodes have ensheathed they are again washed, using tap or distilled water, to which they are then isotonic.

The ensheathed nematodes may be kept in water in the bottles for an indefinite period, provided they are occasionally washed. Ordinarily this storage period does not exceed 4 or 5 weeks, and the volume of culture is adjusted to the field demands within such limits. Nematodes have been experimentally stored for as long as 6 months.

The repeated washing of large numbers of the nematodes is tedious and laborious when done by the sedimentation method. A mechanical washing machine has been devised which greatly reduces the time and effort required in cleaning the nematodes. This machine consists of a cylindrical screen of fine mesh (350×20 per inch) wire filter cloth which is rotated rapidly in the nematode suspension, the latter being contained in a cylindrical glass vessel. The openings in the screen are too small to permit the passage of the nematodes, but not small enough to retain the finely divided culture debris. The rotation of the screen prevents a mat of nematodes from forming on the screen surface as the fluid and finely divided material in suspension is withdrawn under a partial vacuum. Fresh wash solution is continuously introduced to replace the dirty solution removed. The machine is capable of thoroughly washing as many as 100 million nematodes within 20 minutes, and the loss of nematodes is less than 0.1 per cent.

FIELD EXPERIMENTS WITH THE NEMATODE

This nematode was discovered in dead Japanese beetle larvae found during a survey at Tavistock Golf Course near Haddonfield, N. J., in May, 1929 (9). Since that time careful surveys have been conducted at several hundred locations in all sections of New Jersey where the beetle population has been heavy. Naturally existing colonies of the parasite have not been found at any of these locations. It therefore appears that the natural distribution of the nematode is decidedly limited. The ability of the parasite to become established in areas where it does not occur naturally is accordingly very important. Experiments directed toward determining this ability have been in progress since 1931. Seventy-three different experimental plots have been treated at various seasons, in all kinds of agricultural soils. Extremes of soil moisture have been encountered, as have widely different schedules of liming and fertilization. In each experiment, soil samples were examined approximately two weeks after treatment. Parasitized beetle grubs were recovered

from 72 of the 73 experiments surveyed. The percentage of the total population parasitized ranged from 0.3 to 81 per cent, depending on soil moisture, nematode dosage, soil temperature, and density of beetle larva population. The consistent success of attempts to establish the parasite indicates that little difficulty should be experienced in accomplishing a wide distribution by introduction of the organism into areas which it has not infested previously.

Two recently adopted practices have resulted in improved field performance of the parasite. When ensheathed nemas were substituted in field treatments for nematodes direct from culture, much higher rates of parasitism were obtained. All introductions are now made using nematodes reared on the veal medium and ensheathed as described above.

The other change in field technique is the substitution of the surface method of application for the previously employed sub-surface method. Under most conditions, more satisfactory results can be obtained when a given number of nemas are distributed continuously over the surface of the area to be treated, than when the same number of nemas are placed beneath the turf in heavy concentrations at spots over the area. In surface application the nemas are applied in water suspension from a sprinkling can or tank sprayer. A sprinkling with clear water follows the application of the nematode suspension.

The ability of the parasite to maintain itself in an area where it has recently been established is also important. Glaser (1) described four small field plots established in southern New Jersey in May 1931. They were treated by the surface application of 27,000 nemas per square foot. The study of these plots has been continued since that time, and parasitized beetle larvae have been recovered each spring and fall since treatment. Healthy beetle larvae have been placed in the frames each spring and fall, but no nematodes have been added since treatment. Hence, the parasite has survived under field conditions for eight and one-half years, with a host population maintained by the periodic addition of healthy beetle larvae.

Another field experiment described by Glaser and Farrell (10) is still in progress. The field was treated May 23–29, 1933, by the subsurface introduction of agar-reared nemas direct from culture. Heavy initial parasitism occurred, followed by a rapid spread of the parasite. Surveys have been conducted in this experiment each spring and fall since treatment, with parasitized grubs being recovered during 17 of the 18 surveys. No parasitism was found in a survey in April, 1938, when the host population dropped to less than one grub per square foot, but parasitized grubs were found in October, 1938, and in May and October, 1939, so the parasite is still active at this location. As indicated by the April, 1938, survey, the Japanese beetle population has been low in this

area for the past few years, not having exceeded 5.4 grubs per square foot since September, 1934. Despite this low host population, the parasite has maintained itself under natural field conditions for six and one-half years.

Many additional demonstrations of the ability of the parasite to become established and maintain itself in areas where it does not occur naturally have been provided by other experiments conducted more recently, so that it is now definitely established that this organism tolerates a wide range of soil conditions.

As mentioned above, it has now been proved that the adult beetles can act as a vector in the dissemination of the parasite. Therefore, if a small colony of the parasites is established, the nemas are spread from this colony by the movement of parasitized adult beetles and a general distribution of the parasites over the surrounding area should result. A colonization program is now in progress in New Jersey, in which colonies of the parasite are being established at three and one-half mile intervals over the State, in an attempt to effect a general distribution of the organism.

The nature of this publication prevents the inclusion of detailed descriptions of field procedures, data, and results. Such material will be presented in a publication now being prepared.

SUMMARY

The life history of *Neoaplectana glaseri* on culture media is similar to the life history in nature. The nematode passes through five stages in its life cycle; but the third stage may or may not appear, dependent upon conditions. If the second-stage larva is in an environment favorable for further development, it proceeds directly to the fourth or pre-adult stage. This occurs in the insect hosts as well as on the artificial media. In this case the third larval stage appears to be suppressed, and the nematode completes its life history with three ecdyses. However, if the second-stage larva is in an environment not suited for further development, the larva ceases to grow, empties the alimentary tract, and forms a new cuticle with the third-stage form within. In this condition the larva is a resistant or true infectious form. Ensheathed cultured larvae may be produced experimentally. This has proved to be important for the survival of the parasites when introduced into the field.

Neoaplectana glaseri does not confine its attack to the larvae of the Japanese beetle. Adult Japanese beetles are readily parasitized, when the nematode is present in the soil in which female beetles have entered for oviposition. Beetles may also become infected during the period between the completion of metamorphosis and the escape from the pupal cell. Under field conditions it has been demonstrated that parasitized

adults are capable of disseminating the nematodes. It has recently been found that the nematode is capable of infecting at least twelve other insects some of which are of economic importance.

The methods for culturing *Neoaplectana glaseri* are described in some detail. Field plots established with cultured nematodes in 1931 and 1933 have been continuously studied and it has been found that the introduced parasites persisted. Seventy-three other experimental introductions made more recently have yielded encouraging results insofar as persistence of the parasite and reduction of beetle grubs is concerned.

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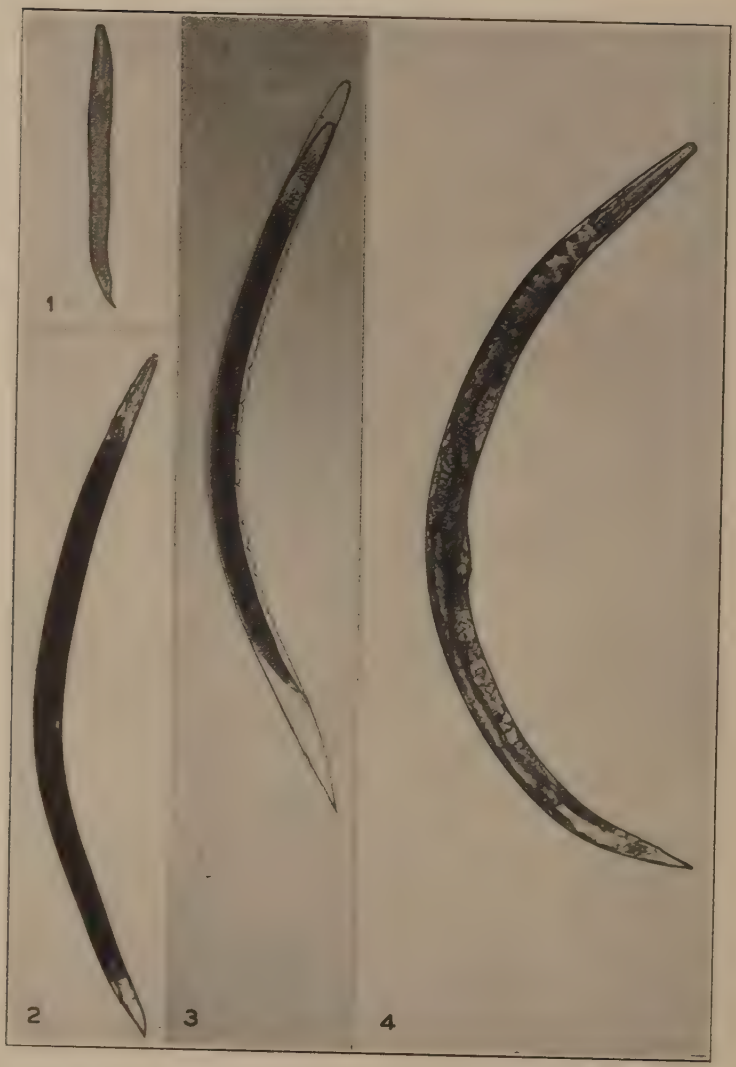

FIG. 1. First-stage *Neoaplectana* larva. ×83.
FIG. 2. Second-stage *Neoaplectana* larva. ×83.
FIG. 3. Ensheathed second-stage *Neoaplectana* larva. ×116.
FIG. 4. Pre-adult *Neoaplectana* female. ×83.
(All photomicrographs by J. A. Carlile.)

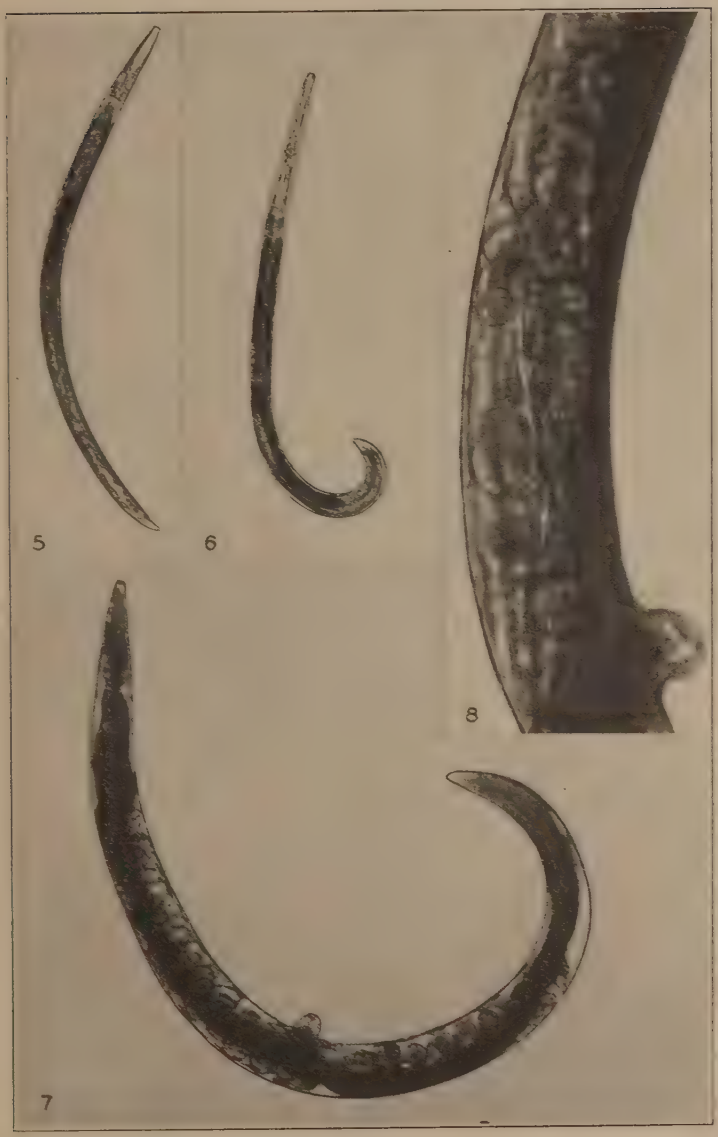

FIG. 5. Pre-adult *Neoaplectana* male. ×62.
FIG. 6. Adult *Neoaplectana* male. ×62.
FIG. 7. Adult *Neoaplectana* female. ×62.
FIG. 8. Section of the body of an adult *Neoaplectana* female. ×170.

THE GENERA *TELORCHIS, PROTENES,* AND *AURIDISTOMUM* (TREMATODA: RENIFERIDAE)[1]

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In the course of ecological studies on the parasites of turtles, many trematodes of the genus *Telorchis* were found. At the suggestion of Dr. Paul D. Harwood[2] a revision of the group was undertaken, which was subsequently extended to the genera *Protenes* and *Auridistomum*. The problem is quite difficult, and will be solved finally only when additional life histories are well enough known to determine with certainty the morphological variations which occur in a single species.

On December 29, 1899, Lühe proposed a new genus of trematode, *Telorchis,* with *T. clava* as type. The following day a paper by Looss appeared in which he proposed the genus *Telorchis,* but with *T. linstowi* as type. The publication date was the same for both papers, December 28, 1899. Braun (1900) recognized Lühe's genus and pointed out that it had priority over *Telorchis* Looss, 1899. The two genera were considered synonymous.

Lühe (1900), after Braun had accepted his genus, made a comparison of the two genera. He divided *Telorchis* into two subgenera, *Telorchis* with *T. clava* as the type and only species and *Cerchorchis* with *T. linstowi* as type. Barker and Covey (1911) added a third subgenus *Protenes*. In 1915 Stunkard rejected Lühe's subgenus *Cercorchis* and raised *Protenes* to generic rank. Perkins (1928) revived *Cercorchis* as a distinct genus, and redefined *Telorchis* and *Cercorchis* while accepting *Protenes*. Dollfus (1929), apparently unaware of Perkins' work, accepted Stunkard's interpretation of the genus. Mehra and Bokhari (1932) proposed a new genus *Paracercorchis* with *P. pellucidus* as type, as intermediate between *Telorchis* and *Cercorchis*. Bennett (1935) gave the first diagnosis of the genus *Protenes*.

The genera *Telorchis, Cercorchis, Protenes,* and *Paracercorchis* all fall into the genus *Telorchis* Lühe, 1899. *Telorchis* according to Perkins differs from *Cercorchis* in the following way: genital pore close to and in front of ventral sucker in *Cercorchis*, genital pore somewhat distant and to the left of the ventral sucker in *Telorchis;* testes in more or less strict

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[2] Drs. H. J. Bennett, A. C. Chandler, P. D. Harwood, F. J. Holl, L. G. Ingles, A. McIntosh, D. B. McMullen, A. S. Pearse, E. W. Price, R. L. Roudabush, H. W. Stunkard, and H. J. Van Cleave all lent specimens for the study, and some contributed valuable suggestions. Their splendid cooperation is greatly appreciated.

tandem at the posterior end in *Cercorchis,* testes oblique or nearly in tandem, midway between the genital aperture and the posterior end in *Telorchis.* These differences are not of generic nature, since in the species *Telorchis robustus* the genital pore varies in position in mounted specimens from the position characteristic of the genus *Cercorchis* to that characteristic of *Telorchis.* The statements that testes are in more or less strict tandem, and testes are oblique or nearly in tandem are practically synonymous, and an examination of the specimen of *Telorchis clava,* the type species of the genus, which MacCallum described as *Plagiorchis anacondae* bears out this contention. The more anterior position of the testes in *Telorchis* is not particularly significant because there is rather a wide variation among species and in individuals of a species in this character. In MacCallum's specimen of *T. clava* the testes are not midway between the genital pore and the posterior end, as Perkins has claimed for the genus *Telorchis,* but are at the junction of the ultimate and penultimate thirds of the distance from the genital pore to the posterior end. Lühe (1899) states that the testes are in the posterior end of the body. The extent of vitellaria is quite different in *T. clava* than in any other species, but as Perkins indicated, this difference is hardly sufficient for the erection of a new genus. For these reasons the genera *Telorchis, Cercorchis* and *Paracercorchis* (which is intermediate between the other two) appear to be superfluous and only *Telorchis* should be recognized.

The genus *Telorchis* Lühe, 1899 needs but one phrase to exclude from it the genus *Protenes.* The diagnosis of the genus *Protenes* by Bennett is perhaps a little too detailed (e.g., length 1.28 to 4.03 mm) to be practical, therefore both genera are here redefined:

Telorchis Lühe, 1899

Diagnosis: Plagiorchioidea: Reniferidae. Elongate, flat distomes with simple oral suckers. Oral and ventral suckers subequal. Pharynx present. Intestinal crura begin preacetabular and end near the posterior end of the body. Vitellaria lateral. Genital pore just anterior to the ventral sucker, usually somewhat to the left of the median line. Cirrus sac extends behind the acetabulum. Ovary posterior to or at the posterior level of cirrus sac. Uterus with descending and ascending coils, does not extend behind the testes. Metraterm anterior to ovary and usually about half as long as cirrus sac. Laurer's canal present. Testes in tandem at the posterior end.

Type species: Telorchis clava (Diesing, 1850).

Protenes Barker and Covey, 1911

Diagnosis: Plagiorchioidea: Reniferidae. Elongate, flat, distomes with simple oral suckers. Oral and ventral suckers subequal. Pharynx present. Intestinal crura start preacetabular and end near posterior end of body. Vitellaria lateral. Genital pore on the left dorsal side in front of the acetabulum. Cirrus sac extends posteriorly to the rear margin of the acetabulum and is well developed. Ovary posterior to the cirrus sac. Uterus with descending and ascending coils, does not extend behind the testes. Metraterm anterior to ovary and usually as long as cirrus sac. Laurer's canal present. Testes in tandem at the posterior end.

Type species: Protenes angustus (Stafford, 1900).

REVISION OF THE SPECIES OF *Protenes*

Barker and Covey (1911) described very completely a worm from the small intestine of *Chrysemys marginata* which they named *Telorchis* (*Protenes*) *leptus*. In the new sub-genus, which was erected to accommodate their new species, they included *Telorchis angustus* Stafford. Following a discussion of the differences which were noted between *P. angustus* and *P. leptus*, Covey (in Barker and Covey, 1911) states, "In view of Stafford's meager description and his indefinite drawing and the differences already noted between *T. angustus* and the form which I have described, I am of the opinion that the two are not the same species and that the form found in *Chrysemys marginata* is a new species." A mounted specimen, in the United States National Museum, of *Protenes* from *Chrysemys picta* was available for study. This specimen was a typical example of *P. leptus*. The difference of host no longer exists. The ovary of *P. angustus* is said to be near the middle of the body, while the ovary of *P. leptus* is near the junction of the anterior and middle thirds of the length of the body. *P. leptus* is wider anterior to the ovary, while *P. angustus* is wider posterior to the ovary. These apparently great differences can be explained if it is assumed that the anterior end of *P. leptus* is contracted and the posterior end extended, thus broadening the portion in front of the ovary and at the same time causing the ovary to be relatively more anterior in position than it would appear in a worm whose anterior end was extended and whose posterior end was contracted as is probably the case with the type specimen of *P. angustus*. In at least one specimen of *P. leptus* which Barker and Covey measured, the ovary has the same position as Stafford indicated for his species. Stafford's specimen is larger than are any of the specimens of *P. leptus* studied by Barker and Covey, but *P. angustus* is exceeded in length by the longest of Barker and Covey's worms and is exceeded in width by the widest. If the measurements of the organs of *P. angustus* are multiplied by the fraction, size of *P. leptus* over size of *P. angustus*, then the sizes of the organs of one species will correspond to the sizes of the organs of the other. In other words the worms have identical proportions. In Stafford's description of *P. leptus* the excretory bladder divides at the level of the posterior testis but in his figure he has shown the division of the excretory bladder to be at the posterior level of the ovary. Stafford failed to mention many organs and also failed to limit the anterior extent of the vitellaria, but since the differences noted by Barker and Covey appear to be too slight to warrant the erection of a new species, and the similarities such as the position of the genital pore, acetabulum, and cirrus sac, are sufficient to show that the worms are very closely related, *P. leptus* is considered to be synonymous with *P. angustus*.

Protenes chapmani Harwood, 1932, from *Pseudemys elegans*, is said

to differ from *P. leptus* Barker and Covey because the genital pore is on the right instead of the left; Laurer's canal extends lateral instead of anterio-lateral as it does in *P. leptus* and there are slight differences in size of body, length of cirrus sac, and the location of the ovary, and acetabulum. An examination of Harwood's specimen showed that the genital pore was near the left margin and not the right. An actual linear difference in the position of the opening of Laurer's canal of 50 microns in a worm 3,000 microns in length, is not sufficient for the erection of a new species. The slight difference in size and position of the organs are within the limits of variation of *P. leptus*.

Protenes vitellosus Bennett, 1935, was likewise taken from *Pseudemys elegans*. Bennett states "*Protenes vitellosus* may be distinguished from *P. leptus*, the species most similar to it, by its much larger size, the absence of a seminal receptacle and the distribution of the vitellaria. In *P. leptus* the vitellaria begin just posterior to the ovary and terminate anterior to the testes, while in *P. vitellosus* the glands extend from the ovary to or posterior to the anterior testis. The eggs of *P. leptus* average 23 by 51 μ, those of *P. vitellosus* 22 by 40 μ." The proportional sizes of the organs of *P. vitellosus* are not very different from those of the other species. Since Bennett's smaller worms are well within the range of those of Harwood, Stafford, and Barker and Covey, his larger specimens will be considered as extending the size range of the species. That Bennett did not see a seminal receptacle is not surprising, since among some twenty specimens of *P. angustus* from *Chrysemys marginata* examined, in only a few worms could this structure be demonstrated. The extent of the vitellaria is somewhat greater than recorded for *P. leptus*. It must be pointed out however that in one specimen of *P. vitellosus* which Bennett identified as such, the posterior extent of the vitellaria corresponded to that given for *P. leptus*. Barker and Covey place the anterior limit of the vitellaria at the ovarian level thus agreeing with Bennett. The difference in the size of the eggs is less convincing than it appears, because Bennett's measurements fall within the extremes of those given by Barker and Covey.

The genus *Protenes* is probably represented only by a single species, *Protenes angustus* (Stafford) 1900. (*Synonyms: Distomum angustum* Stafford, 1900; *Telorchis angustus* (Stafford, 1900) Stafford, 1905; *Telorchis* (*Protenes*) *angustus* (Stafford, 1900) Barker and Covey, 1911; *Telorchis* (*Protenes*) *leptus* Barker and Covey, 1911; *Protenes angustus* (Stafford, 1900) Stunkard, 1915; *Protenes leptus* (Barker and Covey, 1911) Stunkard, 1915; *Protenes chapmani* Harwood, 1932; *Protenes vitellosus* Bennett, 1935.)

REVISION OF THE SPECIES OF *Telorchis*

The genus *Telorchis* is composed of numerous species from a wide

variety of chelonian hosts, as well as from a few snakes and large salamanders. Many of the species are quite similar to each other, in fact so much so that the validity of many is doubtful. In North America, of the 21 species described probably only 12 are distinct. Perhaps when infection experiments are carried out, similar to those of Beaver (1937) who found that the same echinostome could infect various hosts and would appear as several supposed species in different hosts, the number of species of *Telorchis* in North America will be found to be less.

Characters which are to be considered as different and constant enough upon which to determine species are: extent of vitellaria, position of ovary, relative length of cirrus sac, relative length of metraterm, size of suckers, position of acetabulum, size of pharynx, length of esophagus, extent of intestinal crura, and special features such as the presence of a muscular bulb on the metraterm or the presence of small lappets on the oral sucker.

The extent of the vitellaria is a very variable character. In *Telorchis robustus* it varies posteriorly from the level of the anterior testis to a point about midway between the ovary and testes and anteriorly from a level just anterior to the base of the metraterm, to the acetabulum. In *T. medius* the anterior limit varies only within the ovarian level; the variation of the posterior limit is much greater. In *T. cryptobranchi* the anterior limit of the vitellaria is usually in advance of the acetabulum. The species of *Telorchis* can be divided into three groups on the basis of the extent of the vitellaria: (1) in which they do not extend anterior to the ovarian level, (2) in which they extend only as far as the posterior level of the acetabulum, and (3) in which they extend anterior to the acetabulum.

The position of the ovary varies greatly with the amount of contraction and expansion in the different regions of a worm, as was shown for *Protenes*. In spite of this artificial variation the genus can be separated into three more or less distinct groups in regard to the relative position of the ovary. A few species have the ovary very close to the acetabulum, the distance being not more than two ovarian diameters removed from it. The second group is characterized by having the ovary distinctly nearer to the acetabulum than to the testes, but usually more than three ovarian diameters from it. In the third group the ovary lies about midway between acetabulum and testes.

The length of the cirrus sac is quite variable. Its anterior limit is the genital pore, or very near the genital pore in species which have a common genital atrium; its posterior limit varies from one acetabular diameter behind the acetabulum, to the ovarian level. The genus can be separated into two groups: (1) in which the posterior limit of the cirrus sac is at or close to the ovarian level; and (2) in which the posterior limit of

the cirrus sac is distant from the ovary. In many species the posterior position of the cirrus sac varies from a point slightly anterior to the ovary to a point slightly posterior to it. This variation probably occurs in individual worms and is dependent upon the state of contraction of the cirrus sac and of the whole body.

In some species the metraterm is as long as the cirrus sac; in others it is only about half as long. When eggs are crowded into the metraterm it is sometimes difficult to distinguish it from the uterus.

A few telorchids have ventral suckers which are considerably larger than their oral sucker; these are placed in one group. The remainder of the species in the genus *Telorchis* have oral suckers that are slightly larger than, or equal to, the ventral suckers; these comprise a second group. The size of the suckers is of course dependent upon their state of contraction or expansion. Perhaps this character is a poor one for the separation of species, small differences are certainly not significant; but if the two suckers appear to be about equally expanded or contracted, then perhaps a good comparison can be made.

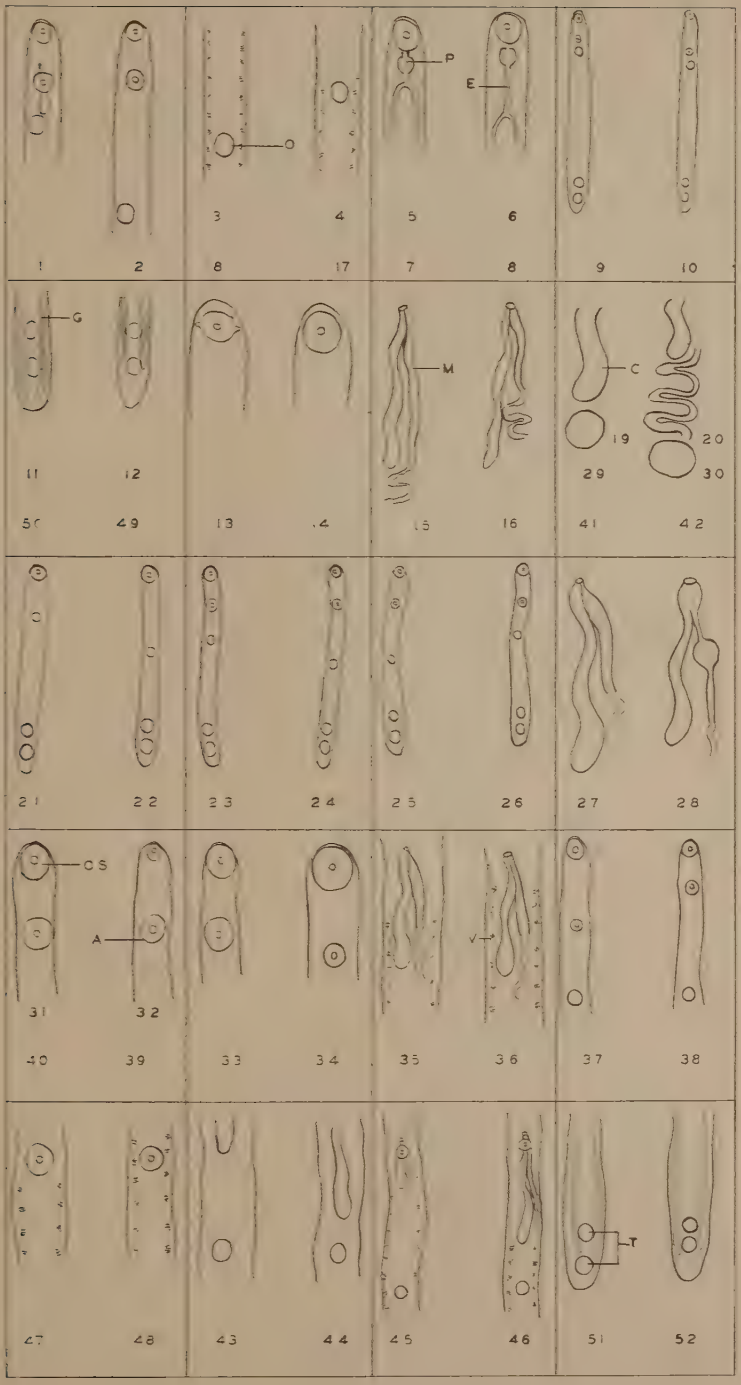
The position of the acetabulum, size of the pharynx, length of esophagus, and extent of the intestinal crura are all variable to a very large degree, within a species. It is therefore impossible to divide the genus into groups based on these characters, but nevertheless these can be used to separate one species from another in particular cases.

Of all the characters available for use in the separation of species, those which are present in only one or two are most satisfactory. These certainly should be used for specific determinations.

A key for the determination of the species for the genus *Telorchis* has been made in which are included only those species which are considered to be valid. Following the key, an alphabetical list of the valid species with their synonymies is given, along with a discussion of each.

FIGS. 1–52. Differential characters of the key. The numbers under each sketch refer to the key, if the diagram and the specimen correspond then the statement is true. (Free-hand diagrammatic drawings.)

A — Acetabulum
C — Cirrus sac
E — Esophagus
G — Gut
M — Metraterm
O — Ovary
OS — Oral sucker
P — Pharynx
T — Testes
V — Vitellaria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KEY TO THE SPECIES OF THE GENUS *Telorchis*

(Figs. 1–52)

1 (2) Ovary not more than two ovarian diameters posterior to acetabulum 3
2 (1) Ovary more than two ovarian diameters behind acetabulum, usually more than three 13
3 (4) Vitellaria extend anterior to ovary 5
4 (3) Vitellaria post-ovarian 7
5 (6) Esophagus shorter than pharynx or absent: *T. clava*.
6 (5) Esophagus longer than pharynx: *T. patonianus*.
7 (8) Esophagus shorter than pharynx or absent 9
8 (7) Esophagus longer than pharynx 11
9 (10) Distance from ovary to testes two thirds of body length: *T. hagmanni*.
10 (9) Distance from ovary to testes one half of body length: *T. pellucidus*.
11 (12) Intestinal crura extend posterior to the testes: *T. stammydis*.
12 (11) Intestinal crura end in the testicular zone: *T. parvus*.
13 (14) Oral sucker with small lappets 15
14 (13) Oral sucker without small lappets 17
15 (16) Metraterm as long as cirrus sac: *T. bifurcus*.
16 (15) Metraterm shorter than cirrus sac: *T. auridistomi*.
17 (18) Vitellaria do not extend an ovarian diameter beyond the anterior margin of the ovary 19
18 (17) Vitellaria extend beyond an ovarian diameter anterior to the margin of the ovary 25
19 (20) Posterior end of cirrus sac over ovary or very near to it 21
20 (19) Posterior end of cirrus sac separated from ovary: *T. medius*.
21 (22) Ovary anterior to the middle of the body 23
22 (21) Ovary at the middle of the body: *T. diminutus*.
23 (24) Distance from ovary to acetabulum one-sixth of body length: *T. pleroticus*.
24 (23) Distance from ovary to acetabulum one-fourth of body length: *T. pseudoaculeatus*.
25 (26) Ovary about midway between acetabulum and testes 27
26 (25) Ovary nearer to acetabulum than to testes 39
27 (28) Without a muscular bulb on metraterm, no common genital sinus 29
28 (27) With a muscular bulb on metraterm and a common genital sinus: *T. singularis*.
29 (30) Cirrus sac reaches ovarian level 31
30 (29) Cirrus sac extends not more than two-thirds of the distance from genital pore to ovary 37

31 (32) Ventral sucker not larger than oral sucker 33
32 (31) Ventral sucker larger than oral sucker: *T. solivagus.*
33 (34) Esophagus usually absent, oral and ventral suckers subequal 35
34 (33) Esophagus present, oral sucker almost twice as large as ventral sucker: *T. stossichi.*
35 (36) Vitellaria end at posterior level of metraterm: *T. dhongokii.*
36 (35) Vitellaria extend beyond posterior level of metraterm: *T. robustus.*
37 (38) Acetabulum half way between oral sucker and ovary: *T. konoi.*
38 (37) Acetabulum nearer to oral sucker than to ovary: *T. attenuatus.*
39 (40) Ventral sucker larger than oral sucker 41
40 (39) Ventral sucker not larger than oral sucker 47
41 (42) Cirrus sac reaches ovary or is not separated from it by uterine coils: *T. stunkardi.*
42 (41) Cirrus sac separated from ovary by uterine coils 43
43 (44) Posterior end of cirrus sac more than an ovarian diameter anterior to ovary 45
44 (43) Posterior end of cirrus sac not more than an ovarian diameter anterior to ovary: *T. necturi.*
45 (46) Cirrus sac ends at a level one-third of the distance from ovary to acetabulum behind ventral sucker; vitellaria extend to posterior end of metraterm: *T. geoclemmydis.*
46 (45) Cirrus sac ends at a level two-thirds of the distance from ovary to acetabulum behind ventral sucker; vitellaria extend only as far as posterior end of cirrus sac: *T. cyclemidis.*
47 (48) Vitellaria do not extend beyond acetabulum 49
48 (47) Vitellaria extend beyond acetabulum: *T. cryptobranchi.*
49 (50) Intestinal crura do not extend beyond testes 51
50 (49) Intestinal crura extend beyond testes 53
51 (52) Crura end in intertesticular zone: *T. poirieri.*
52 (51) Crura end at posterior margin of posterior testis: *T. sirenis.*
53. Several species which can only be separated at present by host and geographical distribution.

	Hosts	*Locality*
a. *T. ercolanii*	Snakes	Europe, Africa, and Asia Minor
b. *T. corti*	Turtles	North America
c. *T. gabesensis*	Clemmys	Africa, Tunisia
d. *T. aculeatus*	Testudo	Europe and Asia Minor

DISCUSSION OF THE SPECIES OF *Telorchis*

Telorchis aculeatus (von Linstow, 1879)

Synonyms: Monostomum aculeatum von Linstow, 1879; *Distomum linstowi* Stossich, 1890; *Telorchis linstowi* (Stossich, 1890) Looss, 1899; *Cercorchis linstowi* (von Linstow, 1879) Perkins, 1928.

T. aculeatus has the vitellaria pre-ovarian, but post-acetabular: the ovary closer to the acetabulum than to the testes; the cirrus sac near the ovary; the metraterm shorter than the cirrus sac; and the ventral and oral suckers sub-equal. This arrangement of the anatomy of *T. aculeatus* places it very close to *T. ercolanii, T. corti,* and *T. gabesensis.*

Host: Testudo graeca.

Locality: Europe and Asia Minor.

Telorchis (?) *arrectus* (Molin, 1859)

T. (?) *arrectus* is probably not a member of the genus *Telorchis,* since the uterine coils are said to extend behind the posterior testis. Dollfus (1929) believes that it might be closely related to *Lepoderma mentulatum* (Rudolphi).

Host: Lacerta muralis.

Locality: Europe.

Telorchis attenuatus Goldberger, 1911

Synonyms: Telorchis (*Cercorchis*) *attenuatus* Goldberger, 1911; *Cercorchis attenuatus* (Goldberger, 1911) Perkins, 1928.

T. attenuatus has the vitellaria pre-ovarian but post-acetabular; the ovary midway between acetabulum and testes; the cirrus sac distant from the ovary; the metraterm shorter than the cirrus sac; ventral and oral suckers sub-equal. In regard to these characters, it is identical with *T. konoi,* but the two species can be separated by the position of the acetabulum. In *T. konoi* the acetabulum is midway between the oral sucker and the ovary, while in *T. attenuatus* the acetabulum is much nearer to the oral sucker than to the ovary.

Hosts: Chrysemys marginata, Chrysemys picta.

Locality: North America.

Telorchis auridistomi (Byrd, 1937)

Synonym: Cercorchis auridistomi Byrd, 1937.

T. auridistomi has vitellaria beginning at the ovarian level; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm less than half as long as cirrus sac; and oral sucker larger than ventral sucker. *T. auridistomi* shares the above characteristics with *T. pseudoaculeatus;* but the two species can be separated because *T. auridistomi* has lappets on the oral sucker, while *T. pseudoaculeatus* does not.

Host: Farancia abacura.

Locality: North America.

Telorchis bifurcus (Braun, 1899)

Synonyms: Distomum bifurcum Braun, 1899; *Cercorchis bifurcus* (Braun, 1899) Perkins, 1928.

T. bifurcus has the vitellaria post-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm as long as cirrus sac; oral and ventral suckers sub-equal. *T. bifurcus* agrees with *T. auridistomi* in having small lappets on the oral sucker, but differs from it in the length of the metraterm.

Host: Podocnemis expansa.

Locality: South America.

Telorchis clava (Diesing, 1850)

Synonyms: Distoma clava Diesing, 1850; *Plagiorchis anacondae* MacCallum, 1921; *Telorchis anacondae* (MacCallum, 1921) Perkins, 1928.

T. clava, the type species of the genus, has the vitellaria anterior to the acetabulum; ovary very near the acetabulum, cirrus sac near ovary; metraterm as long as cirrus sac; and oral sucker larger than ventral sucker. *T. clava* is the only mem-

ber of the genus which has such a combination of characters. Odhner (1926) pointed out that *Plagiorchis anacondae* MacCallum, 1921 is synonymous with *Telorchis clava.*

Hosts: Eunectes murinus, Zamenis constrictor, Oxyropus cloelia, Oxyropus fasciatus.

Locality: South America.

Telorchis clemmydis (Yamaguti, 1933)

Synonyms: Cercorchis clemmydis Yamaguti, 1933; *Paracercorchis megacotyle* Fukui and Ogata, 1934.

T. clemmydis has the vitellaria restricted to the post-ovarian zone; the ovary very near the acetabulum; the cirrus sac near ovary; the metraterm as long as the cirrus sac; oral and ventral suckers sub-equal. This species is closely allied with *T. pellucidus* from which it can be separated by the fact that it has a long esophagus. *P. megacotyle* has been made synonymous with *T. clemmydis* since these species can not be separated by any morphological differences which seem adequate to the author. They are both from the same host and locality.

Hosts: Clemmys japonica, Geoclemmys reevesi.

Locality: Japan.

Telorchis corti Stunkard, 1915

Synonyms: Telorchis linstowi Goldberger, 1911, (nec Stossich, 1890); *Telorchis lobosus* Stunkard, 1915; *Telorchis insculpti* MacCallum, 1918; *Telorchis guttati* MacCallum, 1918; *Telorchis chelopi* MacCallum, 1918; *Telorchis pallidus* MacCallum, 1918; *Telorchis angustus* MacCallum, 1921, (nec Stafford, 1900); *Cercorchis corti* (Stunkard, 1915), Perkins, 1928; *Telorchis stenoura* Ingles, 1930; *Cercorchis texanus* Harwood, 1932; *Cercorchis medius* McMullen, 1934, (nec Stunkard, 1915).

T. corti, as well as *T. aculeatus, T. gabesensis,* and *T. ercolanii,* has vitellaria post-acetabular but pre-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm shorter than cirrus sac; ventral and oral suckers sub-equal. All of the species which have been considered synonymous with *T. corti* also have these characteristics. *T. lobosus* and *T. pallidus* are young undeveloped worms. A specimen of *T. pallidus* which was identified by MacCallum was examined and found to be extremely delicate, with relatively few eggs. Some young specimens from the snapping turtle were found to have the lobed testes for which *T. lobosus* is named. The other species have been described from adult specimens, each author having found differences to exist between his species and *T. corti.* These differences, are not considered to be sufficient for the creation of new species, in the light of wide variation which is shown to be present within a species of the genus.

In the discussion of the genus *Protenes,* apparent specific differences have been explained in some detail to be unimportant. Here no attempt will be made to discuss each species. Measurements, based upon determinations of 64 worms from nine of the ten host species reported as harboring *T. corti* are given in Table 1.

McMullen (1934) worked out the life history of a telorchid which was parasitic in *Chrysemys picta.* He reported this worm as *T. medius,* but his drawings and description show it to be *T. corti,* as defined here. *T. medius* has never been found in *Chrysemys;* it is known as an exclusive parasite of the Kinosternidae, with the exception of a single record from *Terrapene bauri.* Goldberger (1911) reported *T. linstowi* from *Chelydra serpentina* and hence, since *T. corti* and *T. aculeatus* (= *T. linstowi*) are morphologically similar and since *T. corti* is a common parasite of *C. serpentina,* it is supposed that Goldberger was referring to *T. corti.*

Hosts: Chelydra serpentina, Chrysemys marginata, Chrysemys picta, Clemmys guttata, Clemmys insculpta, Clemmys marmorata, Deirochelys reticularia, Graptemys geographica, Malaclemys macrospilota, Pseudemys elegans.

Locality: North America.

Telorchis cryptobranchi (McMullen and Roudabush, 1936)

Synonym: Cercorchis cryptobranchi McMullen and Roudabush, 1936.

TABLE 1.—*Average measurements, in mm, of* Telorchis corti *from various hosts*

Host	*Deirochelys reticularia*	*Clemmys marmorata*	*Pseudemys elegans*	*Malaclemys macrospilota*	*Chelydra serpentina*	*Graptemys geographica*	*Clemmys insculpta*	*Chrysemys picta*	*Chrysemys marginata*
Number of specimens	1	10	17	1	21	4	2	7	5
Length	4.93	4.28	4.21	4.20	4.00	3.32	3.11	2.60	1.60
Width	.54	.44	.39	.54	.46	.32	.86	.33	.10
Diam. oral sucker	.07	.13	.13	.18	.13	.12	.25	.09	.11
Length pharynx		.06	.08	.00	.05	.06	.07	.03	.05
Length esophagus		.15	.14	.11	.14	.06	.05	.06	.05
Diam. ventral sucker	.06	.10	.10	.14	.08	.08	.15	.07	.08
Edge of ventral sucker to level genital pore	.04	.06	.08	.07	.08	.02	.05	.03	.06
Midline to genital pore	.01	.04	.08	.06	.04	.05	.14	.05	.05
Oral sucker to ventral sucker	.74	.73	.77	.88	.74	.60	.45	.29	.32
Length metraterm	.32	.41	.37	.45	.48	.16	.38	.20	.26
Length cirrus sac	.92	.72	.87	1.09	.82	.68	.59	.38	.39
Ant. vitellaria to center level of ventral sucker on left	.23	.68	.73	.54	.42	.51	.09	.21	.008
Ant. vitellaria to center level of ventral sucker on right	.18	.16	.53	.77	.43	.53	.09	.21	.07
Diam. ovary	.16	.11	.12	.25	.12	.11	.16	.09	.07
Ovary to cirrus sac	.06	.23	.25	.05	.12	.13	.15	.07	.07
Ovary to ventral sucker	.93	.91	1.04	.92	.77	.79	.47	.10	.28
Ovary to anterior testis	2.16	1.86	2.05	1.26	1.40	1.49	1.86	1.01	.73
Diam. anterior testis	.16	.19	.17	.29	.18	.16	.28	.15	.12
Distance between testes	.07	.08	.08	.02	.05	.05	.03	.03	.03
Diam. posterior testis	.09	.18	.16	.20	.18	.15	.31	.15	.12
Vitellaria to anterior testis on left	.63	.79	.55	.05	.25	.42	.45	.26	.09
Vitellaria to anterior testis on right	.95	.71	.65	.09	.35	.40	.54	.33	.13
Testis to posterior end	.32	.36	.33	.45	.29	.25	.25	.24	.10
Testis to end of gut on left		.26	.24	.36	.20	.11	.18	.15	.07
Testis to end of gut on right		.25	.24	.34	.20	.16	.22	.14	.07

TABLE 2.—*Average measurements, in mm, of* Telorchis medius, T. attenuatus, T. cryptobranchi *from various hosts*

Host	*Telorchis medius*				*Telorchis attenuatus*		*Telorchis cryptobranchi*
	Kinosternon louisianae	*Sternotherus carinatus*	*Terrapene bauri*	*Kinosternon steindachneri*	*Chrysemys picta*	*Chrysemys marginata*	*Cryptobranchus alleganiensis*
Number of specimens	5	1	5	4	4	9	10
Length	3.34	2.39	2.20	1.83	12.02	6.44	3.56
Width	.34	.27	.31	.22	.68	.56	.50
Diam. oral sucker	.10	.09	.09	.06	.16	.13	.12
Length pharynx	.05	.05	.04	.02	.11	.07	.05
Length esophagus	.17	.10	.07	.11	.14	.14	.09
Diam. ventral sucker	.10	.07	.09	.06	.13	.10	.11
Edge ventral sucker to level genital pore	.05	.02	.04	.04	.06	.10	.06
Midline to genital pore	.04	.03	.04	.08	.04	.04	.05
Oral sucker to ventral sucker	.63	.36	.31	.34	1.70	1.04	.45
Length metraterm	.22	.14	.27	.09	1.00	.51	.32
Length cirrus sac	.56	.36	.45	.31	2.27	1.08	.52
Ant. vitellaria to center level of ventral sucker on left	.43	.36	.47	.32	.88	.71	.03
Ant. vitellaria to center level of ventral sucker on right	.51	.38	.48	.38	1.04	.69	.02
Diam. ovary	.15	.11	.09	.06	.26	.20	.11
Ovary to cirrus sac	.12	.11	.03	.09	1.89	.84	.08
Ovary to ventral sucker	.61	.34	.50	.34	4.26	1.77	.45
Ovary to anterior testis	1.43	1.01	.76	.65	4.52	2.67	1.57
Diam. anterior testis	.15	.13	.12	.07	.39	.20	.20
Distance between testes	.01		.02	.03	.17	.09	.03
Diam. posterior testis	.15	.12	.12	.08	.35	.16	.21
Vitellaria to anterior testis on left	.34	.14	.17	.20	.60	.91	.33
Vitellaria to anterior testis on right	.23	.09	.19	.22	.57	.86	.33
Testis to posterior end	.22	.11	.18	.17	.53	.31	.23
Testis to end of gut on left	.14	.09	.08	.07	.44	.25	.15
Testis to end of gut on right	.07	.14	.09	.08	.46	.26	.15

T. cryptobranchi has vitellaria pre-acetabular; ovary nearer to acetabulum than to testes; cirrus sac near to ovary; metraterm shorter than cirrus sac; and acetabulum and oral suckers sub-equal. This is closely related to *T. stunkardi* but differs from it in that the vitellaria are more anterior in *T. cryptobranchi.* Measurements of specimens of *T. cryptobranchi* are included in Table 2.

Host: Cryptobranchus alleganiensis.

Locality: North America.

Telorchis cyclemidis (Tubangui, 1933)

Synonym: Cercorchis cyclemidis Tubangui, 1933.

T. cyclemidis has vitellaria pre-ovarian but post-acetabular; ovary nearer to acetabulum than to testes; cirrus sac distant from ovary; metraterm much shorter than cirrus sac; acetabulum larger than oral sucker. This worm is similar in general structure to *T. corti* but differs from it in the position of the cirrus sac and the size of the suckers.

Host: Cyclemis amboinensis.

Locality: Philippine Islands.

Telorchis dhongokii (Mehra and Bokhari, 1932)

Synonym: Cercorchis dhongokii Mehra and Bokhari, 1932.

T. dhongokii has vitellaria post-acetabular but pre-ovarian; ovary midway between acetabulum and testes; cirrus sac near ovary; metraterm much shorter than cirrus sac, and oral and ventral suckers sub-equal. This worm is very close to *T. robustus,* yet it differs from it by having the vitellaria end at the posterior level of the metraterm.

Host: Kachuga dhongoka.

Locality: India.

Telorchis diminutus Stunkard, 1915

Synonyms: Cercorchis diminutus (Stunkard, 1915) Perkins, 1928; *Cercorchis kinosterni* Byrd, 1936.

T. diminutus has vitellaria post-ovarian; ovary about midway between anterior and posterior ends; cirrus sac near ovary; metraterm about half as long as cirrus sac; oral and ventral suckers sub-equal. Byrd (1936) states that *Cercorchis kinosterni* is closest to *C. bairdii* Harwood. This is quite true, but then *T. diminutus* is also; and a comparison of *C. kinosterni* with *T. diminutus* shows that the two species can not be separated. *C. bairdii* is synonymous with *T. medius,* a parasite of mud turtles. *T. medius* and *T. diminutus* are found in identical hosts, so it is quite possible that *T. diminutus* is a stage in the development of *T. medius.* The author has not had the opportunity to study many specimens of *T. medius* and *T. diminutus,* hence each will be considered as a separate species. Measurements of both species are included in Table 3.

Hosts: Kinosternon subrubrum, Kinosternon louisianae, Kinosternon steindachneri.

Locality: North America.

Telorchis ercolanii (Monticelli, 1893)

Synonyms: Distomum signatum Ercolani, 1881 (nec. Dujardin, 1845); *Distomum ercolanii* Monticelli, 1893; *Telorchis nematoides* (Muhling, 1898); *Cercorchis ercolanii* (Monticelli, 1893), Perkins, 1928.

Vitellaria in *T. ercolanii* are post-acetabular but pre-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm half as long as cirrus sac; ventral and oral suckers sub-equal. Dollfus (1929) gives an excellent account of the variation met with in *T. ercolanii.* He does not make *T. ercolanii* synonymous with *T. nematoides,* even though he found specimens of *T. ercolanii* that coincided with the description of *T. nematoides.* The greatest difference between the two

TABLE 3.—*Average measurements, in mm, of* Telorchis robustus, T. singularis, T. stunkardi, T. diminutus *from various hosts*

Host	*T. robustus*		*T. singularis*			*T. stunkardi*		*T. diminutus*
	Terrapene major	*Terrapene carolina*	*Pseudemys elegans*	*Pseudemys scripta*	*Pseudemys troosti*	*Amphiuma means*	*Amphiuma tridactylum*	*Kinosternon steindachneri*
Number of specimens	5	5	5	1	7	2	4	2
Length	7.62	7.08	9.91	7.43	6.48	3.42	2.47	1.35
Width	.72	.96	.98	.74	.35	.66	.34	.32
Diam. oral sucker	.20	.21	.16	.11	.12	.18	.13	.08
Length pharynx	.09	.11	.12	.09	.06	.07	.06	
Length esophagus	.009	.07	.18	.05	.19	.16	.06	
Diam. ventral sucker	.17	.19	.17	.11	.10	.24	.18	.08
Edge ventral sucker to level genital pore	.13	.09	.07	.02	.06	.06	.07	.009
Midline to genital pore	.05	.06	.03	.09	.06	.08	.06	.03
Oral sucker to ventral sucker	1.24	1.06	1.65	.97	1.50	.84	.56	.13
Length metraterm	1.12	1.08	1.16	1.00	.47	.52	.25	.16
Length cirrus sac	2.30	2.16	2.63	2.27	1.90	.54	.35	.29
Ant. vitellaria to center level of ventral sucker on left	.53	.48	1.02	.63	.90	.20	.09	.19
Ant. vitellaria to center level of ventral sucker on right	.55	.60	1.00	.54	.90	.36	.10	.16
Diam. ovary	.26	.30	.34	.27	.15	.19	.14	.12
Ovary to cirrus sac	.18	.07	.19	.11	.20	.13	.05	.001
Ovary to ventral sucker	1.91	2.03	2.84	2.38	2.06	.53	.30	.20
Ovary to anterior testis	2.90	2.39	3.65	2.59	2.05	1.41	1.00	.47
Diam. anterior testis	.32	.41	.50	.34	.23	.32	.17	.12
Distance between testes	.12	.03	.06	.05	.05		.01	.02
Diam. posterior testis	.33	.38	.43	.33	.22	.28	.16	.13
Vitellaria to anterior testis on left	.31	.47	.24	.14	.42	.23	.14	.14
Vitellaria to anterior testis on right	.35	.39	.11	.09	.38	.18	.14	.10
Testis to posterior end	.34	.33	.36	.25	.27	.26	.25	.18
Testis to end of gut on left	.27	.23	.26	.20	.21	.10	.12	.10
Testis to end of gut on right	.29	.19	.25	.20	.22	.12	.11	.10

worms is the position of the cirrus sac. In *T. ercolanii* the cirrus sac extends over the ovary in only a few specimens, while in *T. nematoides* it extends over the ovary in all of the known specimens. Dollfus states that until the variation of the cirrus sac is known for *T. nematoides*, the two species should be distinct.

Hosts: Tropidonotus natrix, Tropidonotus tessellatus, Tropidonotus viperinus.
Localities: Europe, Asia Minor, Northern Africa.

Telorchis gabesensis Ruszkowski, 1926

Synonym: Telorchis ruszkowski Yamaguti, 1933.

T. gabesensis has vitellaria pre-ovarian but post-acetabular; ovary nearer acetabulum than testes; cirrus sac near ovary, metraterm shorter than cirrus sac; oral sucker larger than acetabulum. This worm is closely allied to *T. aculeatus*, but is found in Africa and not in Europe.

Host: Clemmys leprosa.
Locality: Northern Africa.

Telorchis geoclemmydis (Yamaguti, 1933)

Synonym: Cercorchis geoclemmydis Yamaguti, 1933.

T. geoclemmydis has vitellaria post-acetabular and pre-ovarian; ovary nearer to acetabulum than to testes; cirrus sac distant from ovary; metraterm shorter than cirrus sac; acetabulum larger than oral sucker. *T. geoclemmydis* shares these characteristics with *T. clemmydis* except that the metraterm is relatively longer in the latter.

Host: Geoclemmys reevesi.
Locality: Japan.

Telorchis hagmanni Lent and Freitas, 1937

T. hagmanni has vitellaria post-ovarian; ovary less than two ovarian diameters from acetabulum; cirrus sac near ovary; metraterm as long as cirrus sac; oral sucker larger than ventral sucker. This worm is very closely related to *T. pellucidus* but differs from it, in that the distance from the ovary to the testes is two-thirds of the body length instead of one half.

Host: Podocnemis expansa.
Locality: South America.

Telorchis konoi (Ogata, 1934)

Synonym: Cercorchis konoi Ogata, 1934.

T. konoi has vitellaria post-acetabular but pre-ovarian; ovary midway between testes and acetabulum; cirrus sac distant from ovary; metraterm as long as cirrus sac; with oral sucker larger than ventral sucker. *T. konoi* is very close to *T. attenuatus* but differs from it in the position of the acetabulum.

Host: Geoclemmys reevesi.
Locality: Japan.

Telorchis medius Stunkard, 1915

Synonyms: Cercorchis medius (Stunkard, 1915) Perkins, 1928; *Cercorchis bairdii* Harwood, 1932.

T. medius has vitellaria largely post-ovarian; ovary nearer to acetabulum than to testes; cirrus sac distant from ovary; metraterm shorter than cirrus sac; oral and ventral suckers subequal. *Cercorchis bairdii* likewise has these characteristics. The only important difference between the two species is that the esophagus reported by Harwood is twice as short as that reported by Stunkard. Measurements (Table 2) show that this does not differentiate the two species, hence they are reduced to synonymy.

Hosts: Kinosternon steindachneri, Kinosternon louisianae, Sternotherus odoratus, Sternotherus carinatus, Terrapene bauri.
Locality: North America.

Telorchis necturi (Perkins, 1928)

Synonym: Cercorchis necturi Perkins, 1928.

T. necturi has vitellaria post-acetabular but pre-ovarian; ovary nearer the acetabulum than the testes; cirrus sac separated from the ovary by uterine coils; metraterm shorter than cirrus sac; ventral sucker larger than oral sucker. *T. necturi* resembles the other species of *Telorchis* which infest amphibians, with the exception of *T. sirenis.*

Host: Necturus maculatus.

Locality: North America.

Telorchis patonianus (Caballero, 1935)

Synonym: Cercorchis patonianus Caballero, 1935.

T. patonianus has vitellaria beginning at acetabular level; ovary less than two ovarian diameters from acetabulum, cirrus sac near ovary; metraterm shorter than cirrus sac; oral sucker larger than ventral sucker. This worm is very much like *T. clava,* but differs from it in the length of the esophagus and distribution of the vitellaria.

Host: Dermophis mexicanus.

Locality: Central America.

Telorchis parvus Braun, 1901

Synonym: Cercorchis parvus (Braun, 1901), Perkins, 1928.

T. parvus has vitellaria post-ovarian; ovary less than two ovarian diameters from acetabulum; cirrus sac near ovary; metraterm as long as cirrus sac; oral sucker equal to ventral sucker in size. *T. parvus* is closely related to *T. clemmydis,* but it can be separated from the latter by the fact that its intestinal crura end in the testicular rather than in the post-testicular zone.

Host: Emys orbicularis.

Localities: Europe, Asia Minor, Northern Africa.

Telorchis pellucidus (Mehra and Bokhari, 1932)

Synonym: Paracercorchis pellucidus Mehra and Bokhari, 1932.

T. pellucidus has vitellaria post-ovarian; ovary less than two ovarian diameters behind acetabulum; cirrus sac near ovary; metraterm shorter than cirrus sac; oral and ventral suckers of about the same size. *T. pellucidus* is close to *T. hagmanni* but it can be distinguished from the latter by the position of the ovary.

Host: Kachuga dhongoka.

Locality: India.

Telorchis pleroticus (Braun, 1899)

Synonyms: Distomum pleroticum Braun, 1899; *Cercorchis pleroticus* (Braun, 1899) Perkins 1928.

T. pleroticus has vitellaria post-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm shorter than cirrus sac; oral sucker larger than ventral sucker. *T. pleroticus* is similar to *T. pseudoaculeatus* but differs from it, in that the vitellaria do not extend beyond the posterior margin of the ovary, and that the ovary is nearer the acetabulum.

Host: Turtle.

Locality: South America.

Telorchis poirieri (Stossich, 1895)

Synonym: Distomum gelatinosum Poirier, 1886 (nec Rudolphi, 1819); *Distomum poirieri* Stossich, 1895; *Cercorchis poirieri* (Stossich, 1895) Perkins, 1928.

T. poirieri has vitellaria anterior to the ovary but posterior to the acetabulum; ovary nearer to ventral sucker than to testes; cirrus sac near ovary; metraterm

shorter than cirrus sac; ventral and oral suckers sub-equal. *T. poirieri* is similar to *T. sirenis.*

Host: Emys orbicularis.

Localities: Europe, Asia Minor, Northern Africa.

Telorchis pseudoaculeatus Dollfus, 1929

Synonym: Telorchis aculeatus Stunkard, 1915 (nec von Linstow, 1879).

T. pseudoaculeatus has vitellaria post-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm shorter than cirrus sac; oral sucker larger than ventral sucker. *T. pseudoaculeatus* resembles *T. auridistomi,* but lacks the oral lappets of the latter.

Host: Tropidonotus grahami.

Locality: North America.

Telorchis robustus Goldberger, 1911

Synonym: Cercorchis robustus (Goldberger, 1911) Perkins, 1928.

T. robustus has vitellaria pre-ovarian, but post-acetabular; ovary midway between testes and acetabulum; cirrus sac near ovary; metraterm half as long as cirrus sac; ventral and oral suckers sub-equal. *T. robustus* is similar to *T. dhongokii.*

Hosts: Terrapene carolina, Terrapene major.

Locality: North America.

Telorchis singularis (Bennett, 1935)

Synonyms: Telorchis robustus Stunkard, 1915 (nec Goldberger, 1911); *Cercorchis singularis* Bennett, 1935.

T. singularis has vitellaria pre-ovarian but post-acetabular; ovary midway between acetabulum and testes; cirrus sac near ovary; metraterm shorter than cirrus sac; oral and ventral suckers sub-equal. This worm resembles *T. robustus* but it differs from the latter, in that it has a common genital atrium, and a muscular bulb at the distal end of the metraterm.

Hosts: Chelydra serpentina, Pseudemys elegans, Pseudemys scripta, Pseudemys troosti.

Locality: North America.

Telorchis sirenis (Zeliff, 1937)

Synonym: Cercorchis sirenis Zeliff, 1937.

T. sirenis has vitellaria post-acetabular but pre-ovarian; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm shorter than cirrus sac; oral and ventral suckers sub-equal. *T. sirenis* is closely related to *T. poirieri,* but can be separated from the latter by its longer intestinal crura which do not terminate in the intertesticular zones as is the case in *T. poirieri.* The writer examined some of Zeliff's material which he had deposited in the United States National Museum. The appearance of the worms was such that it left the impression with the author that they had developed under adverse conditions. Many specimens were immature although they had reached the same size as the mature specimens. The mature specimens had rather diffuse reproductive organs and did not seem to be healthy telorchids. Although he has no proof, it is the author's opinion that *T. sirenis* was described from an unnatural host.

Host: Siren lacertina.

Locality: North America.

Telorchis solivagus Odhner, 1902

Synonym: Cercorchis solivagus (Odhner, 1902) Perkins, 1928.

T. solivagus has vitellaria pre-ovarian but post-acetabular; ovary midway be-

tween testes and acetabulum; cirrus sac near ovary; metraterm shorter than cirrus sac; and oral sucker smaller than ventral sucker. This worm is similar to *T. robustus,* but the relative sizes of the suckers are different. Dollfus (1929) has described a subspecies *T. solivagus maroccanus.* If it is found that there are constant differences within a species, which correspond to its host or to its geographical distribution, then perhaps subspecies names would clear up much of the confusion which exists.

Hosts: Clemmys caspica, Clemmys leprosa, Emys orbicularis.
Localities: Europe, Asia Minor, Northern Africa.

Telorchis stossichi Goldberger, 1911

Synonyms: Telorchis poirieri Stossich, 1904 (nec 1895); *Cercorchis stossichi* (Goldberger, 1911) Perkins, 1928.

T. stossichi has vitellaria pre-ovarian but post-acetabular; ovary midway between testes and acetabulum, cirrus sac near ovary; metraterm shorter than cirrus sac; and oral sucker larger than ventral sucker. This worm resembles *T. robustus* and *T. dhongokii* but differs from them by having a definite esophagus and an oral sucker twice the size of the ventral sucker.

Host: Emys orbicularis.
Localities: Europe, Asia Minor, Northern Africa.

Telorchis stunkardi Chandler, 1923

Synonym: Cercorchis stunkardi (Chandler, 1923) Perkins, 1928.

T. stunkardi has vitellaria pre-ovarian but post-acetabular; ovary nearer to acetabulum than to testes; cirrus sac near ovary; metraterm shorter than cirrus sac; and oral sucker smaller than ventral sucker. This species is closely related to *T. cryptobranchi* but it does not have the vitellaria pre-acetabular as does the latter. Measurements of *T. stunkardi* are included in Table 3.

Hosts: Amphiuma means, Amphiuma tridactylum.
Locality: North America.

REVISION OF THE SPECIES OF *Auridistomum*

In 1900 Stafford described a trematode from the gut of *Chelydra serpentina* as *Distomum chelydrae.* Later, in 1905, he created for this species the genus *Auridistomum.* Stunkard (1924) discovered a species of worm, also from the gut of the common snapping turtle, which he named *Pterygotomaschalos attenuatus. Pterygotomaschalos* was differentiated from *Auridistomum* because of differences in body shape, the presence of a prepharynx and esophagus in *Pterygotomaschalos,* and further differences in the location of the acetabulum, ovary and testes. Stafford's description and figure fail to show the prepharynx or esophagus, but specimens of worms which agree with *A. chelydrae* in other respects show both structures.

The differences of body shape and location of the internal organs can be explained. Young specimens of *A. chelydrae* which the author collected from *C. serpentina* were very similar to Stunkard's type specimen which he kindly lent to the writer. This worm is young and the anterior end is greatly elongated. As *A. chelydrae* matures the posterior end becomes relatively larger especially behind the testes where the yolk glands increase in size and number. The numerous eggs which crowd

between the ovary and the testes also change the relative positions of the organs and the shape of the body.

Ralph (1938) described a new genus of trematode *Tetrapapillatrema*. His reason for establishing the new genus was the fact that the worm had four muscular papillae on the oral sucker. Ralph's description and diagrams of the sucker show that it is the same as the sucker on *A. chelydrae*. The relative positions of the organs are also similar to those in *A. chelydrae*.

It is the author's opinion that *Auridistomum, Pterygotomaschalos* and *Tetrapapillatrema* are synonymous, *Auridistomum* Stafford, 1900, having priority. It is also probable that the genus is represented by but a single species: *Auridistomum chelydrae* (Stafford, 1900) Stafford, 1905 (*Synonyms: Distomum chelydrae* Stafford, 1900; *Pterygotomascholas attenuatus* Stunkard, 1924; *Cercaria concavocorpa* Sizemore, 1936; *Tetrapapillatrema concavocorpa* (Sizemore, 1936) Ralph, 1938).

SYSTEMATIC POSITION OF THE GENERA

Byrd and Denton (1938) include the TELORCHIINAE in the RENIFERIDAE of the superfamily PLAGIORCHIOIDEA. This opinion is probably quite sound in view of the similarity of the life histories of members of the genus *Telorchis* to those of *Lechriorchis* and *Zeugorchis*. *Protenes* and *Telorchis* are so similar that there is little doubt that they are very closely related. The fact that two species of the genus *Telorchis* have little lappets on the oral sucker indicates a possible relationship between *Telorchis* and *Auridistomum*. The similarities of structure and life history in the two genera point to a close relationship which is expressed here by including the genus *Auridistomum* in the subfamily TELORCHIINAE along with *Telorchis* and *Protenes*.

SUMMARY

1. The genera *Cercorchis* and *Paracercorchis* are not valid and should be included in the genus *Telorchis*.

2. The genus *Protenes* is distinct from *Telorchis*, and is represented as far as is known by a single species, *P. angustus*.

3. Thirteen of the 43 species of the genus *Telorchis* appear to be invalid.

4. The genera *Pterygotomaschalos* and *Tetrapapillatrema* probably were based upon specimens of *Auridistomum chelydrae*.

5. *Protenes, Telorchis,* and *Auridistomum* are placed in the subfamily TELORCHIINAE, family RENIFERIDAE, superfamily PLAGIORCHIOIDEA.

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REVIEWS

THE PRINCIPLES OF PARASITE CONTROL. By Thomas W. M. Cameron. 48 pages, 3 diagrams. National Business Publications, Ltd., Gardenvale, Quebec. 1940. 50 cents.

The immediate and pressing problem of curing domestic animals of a multitude of ailments and a second problem, the building of the foundations of knowledge which would provide support for an enlightened practice of the future, both have demanded the attention of the veterinary profession. In the realm of parasitology the former function, the role of the practitioner, has held precedence. It has thus come about that, with regard to parasite control, the art rather than the science of veterinary parasitology has grown up. The canons of this art are given by Dr. Cameron in his synopsis, "The Principles of Parasite Control." In that so many of the methods attempted in control of parasites have not complete support of scientific experimental evidence, perhaps the term "practices" would have been preferable to "principles."

To quote the author, "The subject matter represents the substance of three lectures delivered under the auspices of the University of London at the Royal Veterinary College in September 1938." While the primary intention has been to set forth, in a general way, the methods of parasite control, some consideration, at comparative length, has been devoted to an account of experiments carried out at MacDonald College upon the use of chemical means for destroying the free-living stages of sclerostomes.

By way of introduction to those portions of the booklet which, in character, are more directly related to the title, sections on "Parasitism," "The Importance of Parasites" and "The Parasitic Environment" have been included. These sections contain viewpoints with which not everyone will concur. Particularly is this so in reference to the host-parasite relationship. Cameron in his attempt to provide for every conceivable eventuality, has tended to be preoccupied more with the devising of a terminology than with interpretation of known results of research. If one were to proceed from a consideration of his schematic arrangement of the host-parasite relationship to an examination of a population of internal parasites in which only fully functioning adults were found, these known to be a fraction of the invading infective larvae, the investigator would be at a loss to interpret his findings. To "any particular state is true absolutely, not comparatively, for any individual worm" I would append "at one point of time and provided the parasitic cycle does not involve migration through the host." The division of acquired immunity and premunition is accepted by Cameron though there is little in the literature to suggest their being distinct phenomena.

Potential means for the control of parasites are given, each with brief comments. Although no attempt has been made to evaluate the various forms of control it is probable that Canadian problems and conditions have been kept in mind. Reference is made to modifications of normal farm managemental operations which are in common use in the control of stock parasites. Among these, there are some that have still to obtain scientific sanction. For instance, it is doubtful whether the gain derived from moving farm animals every six days has basis entirely in parasitological fact; and so too, for the time-honored procedure of rotating lambs ahead of mature stock.

In places Cameron's enthusiasm for his subject has led him to overrate the importance of parasites; the causative agents in bringing rodent plagues to a close are still being sought; and the suggestion that farmers underestimate the effects of parasites is not in accordance with all veterinary experience, for in sheep-raising countries especially, the presence of helminth parasites has often been clutched upon as an alibi in the diagnosis of diseases of mysterious etiology.

It is recommended that herds should be treated regularly "until parasites have disappeared and no residual infection left to act as a starting point for re-infection." Until there are adequate means of diagnosis and also completely efficient

anthelmintics this advice will be difficult to act upon. Farmers are adjured to keep their stock from "used land" until all larvae are dead. A question here is, how are farmers to know when all larvae are dead.

If, on occasions Cameron be suspected of rationalising, it may be pointed out that the field of literature which he reviews is noteworthy for its intermingling of fact and conjecture so that authors are exposed to the risk of unwittingly perpetuating the fancies of the past. For this reason alone, it would have been a valuable addition to this work to have incorporated a reference list.

In the opinion of the reviewer sections on diagnosis, procedure in anthelmintic treatment, the ways in which parasites produce their effects and what could be told of the factors that play a part in epidemiology would not have been irrelevant.

The vague generalisations that may be found on almost any page make it apparent that veterinary parasitology has lagged behind other branches of applied biology. Thus while the practical problems are often obvious enough the subject is still in the stage where a scientific definition of these problems is awaited. This is clearly seen in the domain of anthelmintics where the bluestone (to which Cameron refers only incidentally), once used to deter small boys from stealing grapes in southern France, still holds important sway. If the search for anthelmintics were to take the form of investigations into the relationship of parasites to their physico-chemical environment it is possible that progress might be accelerated in this corner of the subject. The lack of knowledge on the identity and significance of the factors that govern the process of infection is evident. An ecological approach, just to name one allied science whose services might be solicited, would contribute much information and help illuminate the problems of epidemiology.

Dr. Cameron's simple and straightforward writing, devoid of labored unnecessary forms of analogy sometimes used in other publications of this type, makes easy reading. Perhaps much of the constipated and diarrheic literature of parasitology would not be so dull reading if there were less art in the subject matter and more in its literary expression.—J. H. Tetley.

Human Helminthology. A Manual for Physicians, Sanitarians and Medical Zoologists. By Ernest Carroll Faust. 780 pages, 302 illustrations. Lea and Febiger, Philadelphia. 1939. 2nd edition. $8.50.

Faust's "Human Helminthology," the first edition of which appeared a decade ago, has during that period established itself as one of the standard texts in the field of helminthic parasitology. It will be gratifying to many whose professional interests bring them in contact either directly or indirectly with the helminths of man to have at their disposal the recently issued second edition. Although the textual matter in the new edition covers 97 more pages than in the old, the general form and organization are essentially unchanged. The author has incorporated much of the information on human helminthic infections which has accrued in the interim. This has necessitated extensive re-writing and editing, although many sections are not appreciably changed. The volume has been improved by the addition of new material. Modification or deletion of some of the older information, as well as the correction of certain errors in the original edition, has also enhanced the value of the new text. The inclusion of an author index in this edition and the considerable expansion of the bibliography makes it a very desirable reference volume.

The use of a larger amount of medical terminology concerning the symptomatology and pathology in various helminthiases is regarded by the reviewer as a natural outgrowth, first, of the author's association with a group whose primary interest is in tropical medicine and secondly, of widespread use of the book by practitioners and clinicians in tropical and sub-tropical regions. To this end the inclusion of a chapter on modern anthelmintic treatment seems particularly appropriate. This has in no way detracted from the book from a zoological point of view. The author has not only made desirable changes concerning the taxonomy of helminths, but has conscientiously included much of the recent biological information. Along some lines this has not been done as critically as one could wish and an uninitiated reader may gather impressions which are not justified factually. Specifically the author has

dealt sympathetically with the idea of auto- or hyper-infection and has either stated or suggested that it could occur in the case of several helminths including *Strongyloides*. While the reviewer by no means intends to imply that such is beyond the realm of possibility, he does feel that evidence thereof should be realistic and conform to the precepts of a controlled experiment. Casual postmortem observations seem inadequate to guarantee these requirements. A similar doubt rests against the impression that auto-infection normally occurs in *Hymenolepis nana* as depicted in the diagram on page 296. It is likewise doubtful in the minds of many helminthologists whether the so-called parasitic males of *S. stercoralis* are biologically significant or whether the primitive nematode excretory pore opened into the cloaca (p. 352). The latter is not true of any known form. Prominent because it is repeatedly referred to in the text is the plant nema, *Heterodera radicicola*. This is a synonym of *H. marioni*.

The chapters dealing with the schistosomes especially impressed the reviewer as being excellent. On the other hand, those dealing with the hookworms, while correct insofar as they go, seem not to have canvassed the epidemiological and biological aspects of this problem to the extent justified by available information. The edition is excellently illustrated, many of the figures having appeared in the previous volume. Altogether, Dr. Faust, with able publishing assistance, has produced a commendable, as well as a remarkably complete and readable volume on the subject of human helminthology.—George L. Graham.

Maynard M. Metcalf (1868–1940)

THE CONTRIBUTIONS OF MAYNARD M. METCALF TO PROTOZOOLOGY

Maynard Mayo Metcalf who died on April 19, 1940 at Winter Park, Florida at the age of 72 years, although not a parasitologist, contributed notably to our knowledge of parasitic protozoa. In his early investigations he used gastropods and tunicates but later his researches were devoted almost entirely to ciliated protozoa of the family OPALINIDAE. After publishing at least nine papers on this subject between the years 1909 to 1921, his monograph on The Opalinid Ciliate Infusorians was issued in 1923 by the U. S. National Museum as Bulletin 120. This is a comprehensive work of 484 pages, illustrated by 258 figures. It contains descriptions of the known genera and species, twenty-five that had already been described and one-hundred and twenty that were described for the first time. One of Metcalf's methods of obtaining material from a large number of species of hosts is worthy of note. He was given access to the collection of preserved frogs and toads in the U. S. National Museum. A ventral abdominal incision was made, the rectum slit open and the contents removed for examination. In this way 1,079 specimens were operated upon without detracting from their usefulness for other types of study. The nuclear conditions of the opalinids were described and the relationships among the members of the family discussed.

Perhaps the most interesting phase of Metcalf's studies of the OPALINIDAE was that concerned with the geographical distribution of hosts compared with the host-occurrence and geographical distribution of the parasites. This subject was treated in his monograph of 1923 and again in 1929 in a paper published by the U. S. National Museum entitled Parasites and the Aid They Give in Problems of Taxonomy, Geographical Distribution and Paleogeography. Host-parasite data are presented which indicate: "(1) Genetic relationship between hosts; (2) places of origin and routes of dispersal of both hosts and parasites; (3) ancient land connections between now distinct and widely separated land masses." Examples of the use of such data are as follows:

"The Leptodactylid frogs of tropical America are close relatives of those in Australasia, as shown by the fact that both harbor a peculiar opalinid parasite, *Zelleriella,* and the Australasian zelleriellas are so closely like those of South America that they cannot be distinguished specifically from present descriptions. Leptodactylids have never been in Europe or Asia as indicated by the absence of *Zelleriella* from that continent. The LEPTODACTYLIDAE, therefore, spread from tropical America to Australasia by some southern route, probably by way of Antarctica.

"The tree-frogs (Hylidae) are chiefly tropical American forms, though they are well represented in North America and in Australasia. Only one species, with half a dozen subspecies, is known from Euro-Asia. These Euro-Asian tree-frogs carry a distinctively North American Opalina, thus showing them to be emigrants from North America. Other evidence from Opalina shows, by the way, that this migration occurred since the Middle Pliocene. The Eurasian tree-frogs resemble species in North America and not species in Australasia. The tree-frogs, therefore, reënforce the evidence of the Leptodactylids that there was at one time a southern connection between South America and Australia."

Another monograph by Metcalf is now in press (October 10, 1940) and about ready for distribution by the U. S. National Museum. This monograph is a continuation of that of 1923. It deals largely with South American material which was collected by Dr. Metcalf himself, and includes descriptions of a number of new species, a detailed discussion of the geographical distribution of opalinids and their hosts and a review of the literature since 1923.—Robert Hegner, *The Johns Hopkins University, School of Hygiene and Public Health.*

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